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THE SEASONAL CYCLES OF ASH, CARBOHYDRATE AND NITROGENOUS CONSTITUENTS IN THE TERMINAL SHOOTS OF APPLE TREES AND THE EFFECTS OF FIVE VEGETATIVELY PROPAGATED ROOTSTOCKS ON THEM

II. CARBOHYDRATE FRACTIONS AND LIGNIN

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INTRODUCTION.

THE present paper is the second of the series appearing under the general heading given above. As stated in Part I*, the material used for the work on carbohydrate fractions and lignin was drawn from the same samples as those from which the ash samples were taken. The pomological data, relating to the trees employed and showing the effects of the five rootstocks on growth, are also given in Part I, and reference should be made to these in considering the rootstock relationships to the carbohydrate data.

It will be seen from the data presented here that similar and well defined seasonal cycles of the various constituents occurred in the trees on all the rootstocks, and that the rootstocks did not affect any of the cycles significantly. The latter results thus differ from those for ash constituents where significant differences due to rootstocks were shown.

HISTORICAL.

A detailed survey of the more recent work on the carbohydrate constituents of apple trees was given by the writer in a previous paper (8) on seasonal cycles in apple trees grown under two cultural systems.

Very little work has been carried out on stock and scion chemistry with reference to carbohydrates. The investigations of Colby (2), however, require mention. He analysed a number of scions grafted on Malling Stocks IX and XII and on seedling stocks, several parts of the trees being sampled. Most of his trees were six years old and were lifted in July, though a few varieties were sampled in April and early October.

Colby found that the roots of Malling IX were well supplied with starch, contrary to the idea that the roots of dwarf trees are starved. The introduction of an intermediate stem piece, however, altered the starch content in the roots,

* Journ. Pom. and Hort. Sci., XVI, June 1938, p. 101.

the resulting accumulation or depletion depending on the nature of the intermediate piece.

Upward movement of reserves was limited in early summer in dwarf trees, but not in those on M.XII. Very fruitful trees on M.IX showed early accumulation of starch (mid June-July), while unfruitful, vigorously growing trees on M.XII showed no such accumulation. Other information with regard to nitrogen-carbohydrate relationship in the same trees was also given and has been referred to by Vaidya in Part I of this series.

EXPERIMENTAL.

(1) Field Sampling.

Full details of the method of field sampling are given in Part I. Sampling began in January, the shoots being then about eight months old, and continued until the end of May. The trees were pruned at this point, and the remaining one year shoots were cut back to allow good growth of new shoots, from which the remaining seven months' samples were taken. A picture of the chemical condition of the terminal shoots prior to bud swelling was thus obtained, for comparison with that of new shoot growth from June onwards.

(2) Preservation of Samples.

Preservation of samples in alcohol was carried out as described in the writer's previous paper (8). Duplicates for the carbohydrate analyses were not identical with those for ash and nitrogen, in that they were taken from the freshly cut up material before preservation in alcohol, whilst the latter were not taken till the material had been dried and finely ground.

(3) Methods of Analysis.

The chemical methods used have been described previously (8), with the exception of that for starch. The previous method for starch consisted of malt diastase digestion, followed by acid hydrolysis, converting the products of the enzyme action to glucose, which was then estimated by Schaffer and Hartmann's method. The ground material was autoclaved to break down the cells, previous to enzyme digestion. A criticism of this method is that during the acid hydrolysis, substances other than starch which break down during autoclaving may be hydrolysed and thus give too high a figure for starch content.

In the present work therefore a modification of the method described by Gardner (4) was employed, in order to avoid the acid treatment. Gardner estimated the reducing value of the sugar produced immediately after enzyme hydrolysis of pure starch, using saliva for the enzyme, and he constructed a graph relating the thiosulphate titration values thus obtained to those for corresponding amounts of starch hydrolysed by acid only ; the sugar and starch

were then calculated from the titration figure in the usual way. He employed this standard graph in his analysis of pear tissues. Bish, also, has used a standard graph for taka diastase (1). It is realized that starch in the shoots of trees may not be of exactly the same nature as potato starch, but the latter gives a good basis for a series of comparative estimations such as the present investigations require.

The writer employed malt diastase instead of saliva as used by Gardner, and the digestion was carried out as described in the earlier paper (8) using Walton and Coe's times and temperatures. Dried, purified potato starch was used in preparation of the standard graph. The resulting figures for titration with thiosulphate in the sugar estimation after enzyme digestion, give a straight line when plotted against starch taken, the figures used being shown in Table I,

TABLE I.

Estimation of Potato Starch.

New Method.		Old Method.		
Mg. Starch.	Titration after Enzyme Digestion. Figures used in Standard Graph.	Titration after Enzyme and Acid.	Mg. Starch. found.	Ratio of the two Titrations.
10	1·93	3·86	10·13	2·00
30	5·64	11·33	29·74	2·01
50	9·46	18·91	49·74	2·00
70	13·17	26·04	68·59	1·98
90	17·23	33·47	88·28	1·94

columns 1 and 2. This graph was taken as the standard instead of that described by Gardner. A second experiment was also carried out with similar amounts of starch, taking the digestion after the enzyme action further to glucose, by means of acid, as in the old method, and calculating the starch found. The titration figures (cf. column 3) for this complete conversion were twice the respective figures for malt digestion alone, over the range used in the analysis; the rate decreases slightly with the higher values (cf. column 5). This correspondence between the two titrations and also the calculated starch figures in column 4 confirm the reliability of the method.

One point concerning the method may be mentioned here. When plant material is used, the digested extract does not clear very easily, for it is somewhat colloidal. It was therefore filtered hot, and washed with hot water into a 200 c.c. flask, 2 c.c. of lead acetate solution were added and the mixture cooled and made up to volume. It was then poured into a 400 c.c. beaker containing a little potassium oxalate, and left to stand overnight. Next day it was filtered through dry filter paper and an aliquot part was removed for the sugar determination (cf. Shriner (6)).

When the two methods were tried out on bark and wood, the results for wood were approximately the same for both methods, but the starch figure for bark was reduced by about half by the new method. The order of the figures is the same as that found by Gardner (4). Values for leaves were also found later to be much lower. Further investigation on this point would be interesting, as obviously some constituent of the bark and leaves, which is unaffected by the enzyme, has been hydrolysed on the addition of the acid.

It must be mentioned that Colby (2) makes the following statement with regard to starch estimation. "The writer's observations showed that for work with low starch, high N. succulent shoots, the taka diastase (or malt diastase) method is worthless in starch determination, and even gives 'starch' figures showing trends opposite to the true relationships." No data or details are given in his paper to substantiate this criticism, so that it cannot be further discussed. Colby employed a modification of the methods of Denny (3), Sullivan (7) and Niemann (5), depending on precipitation of starch from an alcoholic extract, with iodine-potassium iodide solution and ammonium sulphate, and subsequent hydrolysis with 2½ per cent. acid.

(4) Agreement between Duplicates.

The data shown below in Table II give the variation of duplicate samples from their means, as calculated from twelve sets taken at random from the analytical results of the investigation. The Standard Error was calculated in the usual way for small samples.

Table II.

Percentage Variation of Duplicate Samples from their Means.

Fraction.	Bark.	Wood.	Leaves.
Alcohol soluble	1·79 ±*0·316*	2·83 ±*0·775*	1·53 ±*0·640*
Reducing sugar ..	2·99 ±*0·787*	2·66 ±*0·910*	7·91 ±*2·011*
Total sugars	2·96 ±*0·670*	3·69 ±*1·180*	5·13 ±*1·826*
Sucrose	5·41 ±*1·334*	18·50 ±*4·347*	14·29 ±*2·111*
Starch	2·87 ±*0·603*	2·55 ±*0·431*	1·95 ±*0·496*
Hemicellulose	0·85 ±*0·284*	1·44 ±*0·338*	2·06 ±*0·577*
Cellulose	2·45 ±*0·370*	3·68 ±*0·699*	Not sampled.
Lignin	1·45 ±*0·451*	1·28 ±*0·335*	,, ,,

It will be seen later that the difference required for statistical significance between the monthly samplings is much greater than these variations between duplicates. The variation for sucrose as seen from the Table is extraordinarily high. This large error disappeared when two duplicate extracts were combined and then divided equally and analysed, showing that the chemical methods of

clearing, hydrolysis and analysis were not at fault. This reduces the source of variation to error of extraction or sampling. The error is also increased by the small amount of sucrose present and the method of difference used in sugar estimation. This variation has been taken into account in the conclusions drawn later, and is also less than the statistical significance figure mentioned above.

RESULTS.

The calculation of results has been made on the basis of residual dry weight, i.e. dry wt. – (wt. of sugars and starch), instead of the total dry weight previously used. This eliminates the effect of fluctuations due to seasonal changes in these carbohydrates and gives slightly higher figures, on the whole, as compared with total dry weights. It was found that the form of the cycles is not altered by this procedure, but some change in the depth of the maxima and minima occurs.

Data relating to the collection of samples, condition of shoots, etc., have been given in Part I.

Owing to lack of space, the graphs showing the seasonal cycles for individual constituents for the five stocks cannot be presented here, but the results for the four stocks M.II, M.V, M.VII and M.B have been averaged and plotted to the same scale in Fig. 1, which shows also the significant changes from month to month. Stock IX was omitted from the graph as samples were obtained for seven months only, viz. Feb. and May-Oct.

Carbohydrate / Nitrogen ratios will be presented in Part III with Nitrogen results.

DISCUSSION.

Seasonal Cycles.

Examination of the curves for the various fractions shows that the form of seasonal cycle for any particular fraction is very similar for all the rootstocks. These cycles will therefore first be considered in general without reference to the stock problem.

They show considerable resemblance to the cycles obtained in the work on Newton Wonder trees (8), hence, in the following discussion the physiological interpretation of the chief features in the seasonal changes will in most cases be omitted in order to avoid repetition. Some comparison with the results of the previous paper will, however, be made here, as the degree of similarity is of considerable interest. Account is taken of the fact that the results of the later experiment are calculated on residual dry weight and the former on total dry weight. Also, there were no data for March and May in the earlier work, and this gives the curves for spring changes a more smooth appearance than was probably correct, judging from the results of the present experiment.

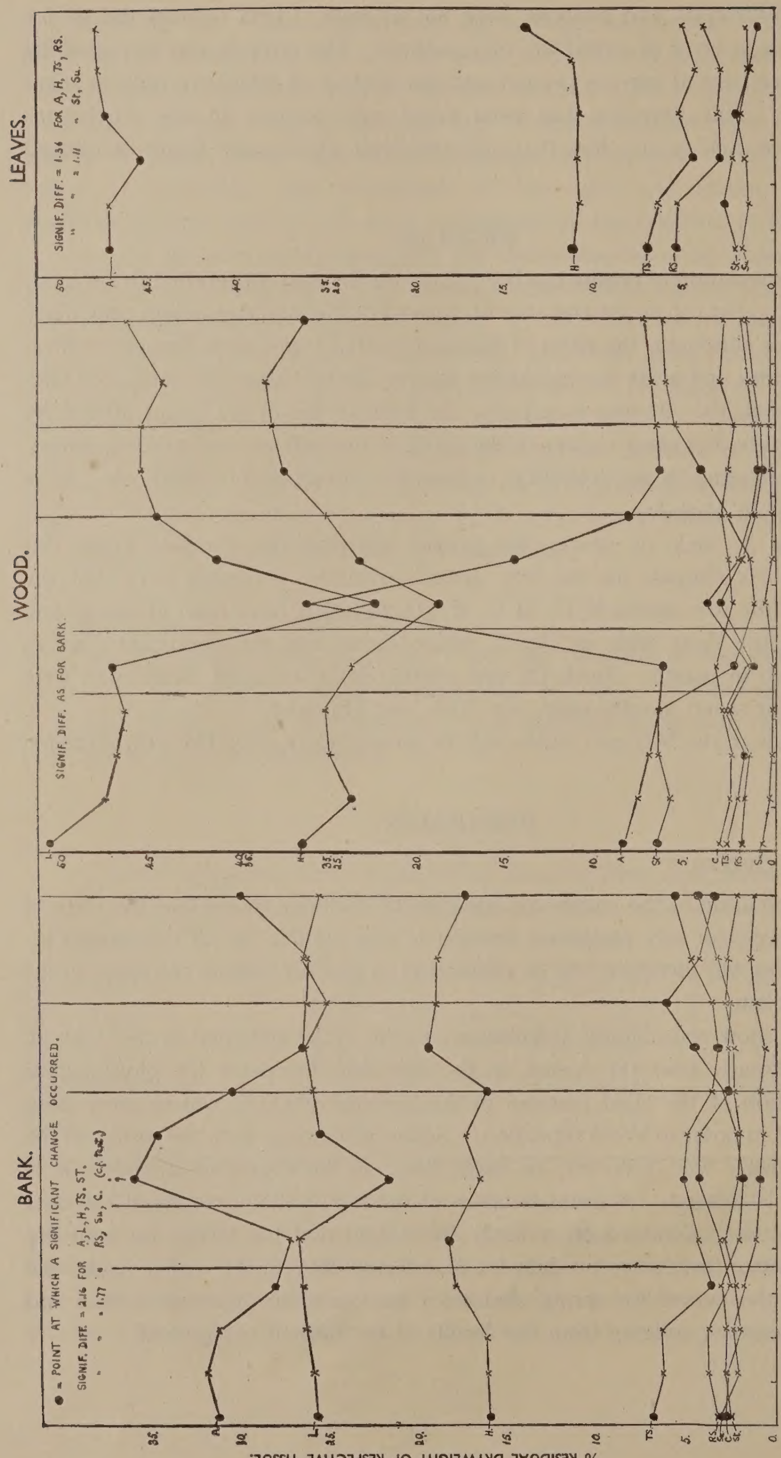

FIG. 1. Average Seasonal Cycles. (Stocks M.II, M.V, M.VII, M.B)

A = ALCOHOL SOLUBLE MATTER. ST. = STARCH. RS = REDUCING SUGARS.
L = LIGNIN. TS. = TOTAL SUGARS. C = CELLULOSE.
H = HEMICELLULOSE. SU. = SUCROSE.

BARK AND WOOD.

(a) *Alcohol Soluble Matter*. The graphs show the usual high value for the young shoots in June in contrast to the low spring figure in the one-year-old shoots, and also the fairly rapid decrease to a similar low figure in the autumn. The general content is, as before, much higher in bark than wood. Average range for Bark = 26·4% Nov.—37·31% June. Wood = 6·05% May—26·41% June (residual dry weight).

(b) *Reducing Sugars*. The main features of cycles are similar to those of the Newton Wonder cycle, though the general level of sugar is higher and the maxima and minima more marked. In the bark there is thus the low spring content, higher June value, and summer decrease with autumnal accumulation, leading however to an earlier maximum in December. In the wood a similar series of changes occurs, but the appearance of the curve is very different from that of Newton, owing to the fact that the June maximum is higher than the maximum in winter. There is also one sharp rise in April. The values range for Bark = 2·55% Oct.—4·43% June. Wood = 0·94% Oct.—3·11% June.

(c) *Sucrose*. In the bark there is a steady fall in spring in the old shoots, a low figure for the new shoots in June, a low summer content and then autumnal accumulation to a high value in January. The curves for the stocks show some variation in December, otherwise the cycles resemble those for Newton. In wood the general content as before is very low, rising to its maximum in January. A small peak for June appears in this later set. Range for Bark = 0·6% Sept.—3·34% Jan. Wood = 0·02% May—0·73% Jan.

(d) *Total Sugar*. These curves are mainly dominated by reducing sugars showing the high June maximum mentioned there in contrast to the Newton Wonder curves. The order of content of the two experiments is approximately the same on the same dry weight basis. Range for Bark = 3·26% Sept.—6·95% Jan. Wood = 0·8% Sept.—3·9% June.

(e) *Starch*. The new starch method lowers the figures for bark by about half. This is evident when comparing the curve with that for Newton. The main characteristics of the two curves are, however, alike, i.e. a June maximum, low summer value, and autumnal accumulation. The maximum is reached, as before, in late October, and is followed by a steep fall, with a January minimum and a slight rise in spring. Thus, the occurrence of a winter minimum in bark is again confirmed. Range for Bark = 1·82% June—6·05% Oct.

Analysis of the wood content by the two methods gave practically the same results in the preliminary experiments. The curves for the cycles by the new method show the general level of starch content to be lower than that for Newton Wonder trees. The same steep fall from spring to June occurs, with

accumulation beginning in midsummer and the content very sharply rising to a maximum, which, however, remains steady for a couple of months instead of decreasing abruptly ; the winter minimum, which occurs in February instead of January, is not so marked. A discussion of the physiological interpretation of the sugar and starch relationships and seasonal changes was given in the former paper (8). Range for Wood = 1·16% June—6·68% March.

(f) *Hemicellulose.* The cycles for this group of substances are again similar to those previously obtained. In the bark the chief points to notice are the low summer content and the rather sharp rise from August to September, as in Newton, with, however, a more gradual decrease and higher spring content than previously observed.

In the wood, the low June value rises sharply to a maximum in October, after which there is a gradual decrease until February. This content is maintained till May, i.e. the last sample of the year-old shoots. The maintenance of a relatively high content in old shoots points to the use of hemicellulose in the cell walls of the plant, since a purely reserve material like starch begins to decrease in the same shoots in April, preparatory to the development of new buds. A similar state of affairs can be observed for the bark.

The general level of values for hemicellulose in bark is higher than that of those for Newton Wonder bark, when the curves are compared on the same dry weight basis. The total carbohydrate content, however, is about the same. These facts, together with the observation that the starch figures are lower, owing to the new method of analysis, confirm the suggested idea of the hemicellulose nature of some of the material previously hydrolysed by the acid after enzyme digestion by the earlier method. This also applies to the leaves, as will be mentioned later. Range for Bark = 16·12% Jan.—19·59% Sept. Wood = 18·99% June—28·63% Nov.

(g) *Reserve and Total Carbohydrates.* The values for reserve carbohydrates are roughly the same for bark and wood owing to the new low starch figures for bark. They show the usual low summer and high winter content with autumnal accumulation. Range for Bark = 6·54% Aug.—9·71% Oct. Wood = 3·34% July—9·47% Jan.

Total carbohydrates reflect the chief points of the starch and hemicellulose curves and do not need fresh comment. Range for Bark = 22·78% Aug.—28·94% Oct. Wood = 24·05% June—36·1% Jan.

(h) *Cellulose and Lignin.* There is nothing of further interest to be discussed from these curves as compared with the earlier work. They show the general uniformity of results for the different stocks. The growth of the young shoots from June onwards is reflected in the lignin curves, which rise steeply from June till mid-August when the lignin content becomes relatively steady.

LEAVES.

(a) *Alcohol Soluble Matter.* A fairly steady high content is maintained throughout the period of sampling. Range=45·6% Aug.—47·6% Sept.

(b) *Reducing Sugars.* This fraction shows the previously obtained decrease from June to early autumn, followed by a rise before leaf-fall. Range=3·22% Aug.—5·55% June.

(c) *Sucrose.* The cycles for sucrose vary somewhat for the different stocks, but indicate a maximum in September, rising from a late summer minimum and decreasing to a low figure previous to leaf-fall. This fraction will be mentioned later in connection with stock differences. Range=0·98% Nov.—2·01% Sept.

(d) *Total Sugars.* These cycles show a high figure for June, as before, and a low value in late summer, rising again when the last sample was taken in November. Range=4·66% Aug.—7·23% June.

(e) *Starch.* The effect of the new starch method is apparent here, resulting in a very much lower level of starch content than occurred for the Newton Wonder trees. The form of the curve from July onwards is almost identical with that previously recorded, showing the decline from July till October and a slight rise before leaf-fall. Range=1·43% Oct.—2·98% July.

(f) *Hemicellulose.* The hemicellulose content is approximately constant until October (when there is a slight rise before leaf-fall) in contrast to the almost continuous rise for Newton Wonder. The general level is also much higher repeating the condition found in the bark, i.e. apparently compensating for the lower starch figure. Range=10·93% July—13·99% Nov.

(g) *Reserve and Total Carbohydrates.* Reserve carbohydrates are lowered, as compared with the Newton Wonder values, by the new starch figures; they show a decrease from June till just before leaf-fall, when there is a rise which reflects the reducing sugar and starch increase at this point. Range=6·1% Oct.—9·4% June.

Total carbohydrates, however, are as high as those of Newton Wonder, confirming the suggestions made in the section on bark results, where higher hemicellulose and lower starch give a total carbohydrate figure similar to that for Newton Wonder. Range=17·4% Oct.—20·66% June.

(h) *Cellulose and Lignin.* These constituents were not estimated in the leaves.

AVERAGED SEASONAL CYCLES.

Fig. 1 shows a graph with the seasonal cycles for the various fractions, averaged for the stocks M.II, M.V, M.VII and M.B (those sampled for twelve months) and plotted to the same scale. Points at which significant changes occurred are marked in black dots. The significant difference was calculated

from analysis of variance tables for four stocks and twelve months. (For stock differences another table taking five stocks for seven months was prepared.) In Fig. 1 two significant difference values are given for each shoot portion, bark plus wood, and leaves. The higher figures, 2·16 for bark plus wood and 1·36 for leaves, are calculated from the Standard Error of difference of two means and Fisher's table. These are used for substances occurring in larger amounts, while the lower figures, 1·77 for bark plus wood and 1·11 for leaves, were obtained by doubling the Standard Error mentioned above, and were employed for sugars and cellulose. This allowed a slightly wider range of significance for these minor fractions. In spite of this, it can be seen from Fig. 1 that the figure 1·77, when applied to the curve for sucrose in wood, is greater than any value in the cycle and is thus a rather severe test for fractions occurring in such small quantities. This arises as a result of grouping of all fractions in one table for purposes of statistical analysis instead of treating them individually. As the uniformity of the results for the five stocks was quite obvious, it was not considered worth while to adopt the latter method.

The cycles are plotted from January, i.e. the dormant season, to make them comparable with those in Parts I and III. The appearance is thus somewhat different from the summary graph for Newton Wonder which begins with the bud swelling period ; but the essential features previously observed can be seen again here.

One point of interest may be mentioned in regard to the degree of continuity of seasonal cycles from year to year. Its discussion was omitted in the earlier paper owing to lack of data for March.

The present experiment began on January 4th, 1934, and the last sample was taken on January 2nd of the following year, 1935. The first five samples before June, when the new shoots appeared, thus belong to one seasonal cycle and the seven after June, to a second and immediately subsequent cycle.

The two January samples represent shoots of similar age but of different cycles. Their internal condition therefore may not be exactly parallel, owing to climatic, cropping and management factors, etc., of their respective years. The first January sample was cut after a normal pruning in the winter of 1933-34 when the leaders were tipped, whilst the second was taken after each of the trees had been deprived of 110 shoots during the eleven previous months of the experiment (ten shoots per tree per sample were removed) following a severe late spring pruning in May 1934 when all terminals were cut back to basal buds.

If the first and last values in the various cycles are examined (Fig. 1), it can be seen that the chemical composition of the shoots of one cycle is not identical with that of the second. Appreciable differences occur in the sugars and starch. Thus total sugars for the second January sample are lower, both

in bark than wood ; starch is somewhat higher for the second sample in bark, but the same in wood ; hemicellulose is slightly higher in bark and the same in wood. These differences suggest that the hydrolysis of the starch had been slightly delayed in the second year.

The difference between one year and another thus appears to be due to change in rate of increase or decrease of a fraction, resulting in a shifting of the date of maxima or minima ; but the similarity between cycles in this experiment and the earlier cycles for Newton Wonder suggest that the nature of a second year's cycle would not be altered to any greater extent.

The results discussed in this section give a useful confirmation of those previously recorded for the ten-year-old Newton Wonder trees on M.II.

In the present investigation, the similarity between the cycles for the five stocks, to be discussed below, suggests that the rootstock has little effect on the carbohydrate content and cycles in terminal shoots of long established trees and that the rate of carbohydrate metabolism is a characteristic unaffected by stock. Moreover, since the general results resemble closely those obtained in the Newton Wonder experiment it would appear that cycles for different varieties may be similar. This point, however, would require further investigation.

STOCK DIFFERENCES.

Only the chemical data need be discussed here, as morphological differences apparent from the sampling data, etc., have been described in Part I.

It is obvious from examination of the graphs of the seasonal cycles that the results for the five stocks taken are strikingly uniform, in bark, wood and leaves respectively. This was confirmed when the data were analysed statistically, and no significant difference was found between the stocks.

With regard to stock IX, however, one point may be mentioned. In the wood, the starch and hemicellulose curves and also total and reserve carbohydrates are considerably higher than those for the other four stocks from June onwards. Although this difference is not statistically significant, the regularity of the curves may permit one to say that there is a tendency for starch and hemicellulose to accumulate during the autumn at a greater rate in the shoots on stock M.IX than in those on M.II, M.V, M.VII and M.B, resulting in a higher maximum during the dormant season.

Colby (2) also noted accumulation from mid-June to July in six-year-old trees on M.IX, but not in comparable trees on M.XII. In the curves just discussed, however, the trees on stocks M.II, M.V., M.VII and M.B also began to accumulate, but to a less extent. The results, however, are not strictly comparable with those of Colby, owing to differences in scion and age of trees.

INTERACTION OF FACTORS.

The following further deductions may be drawn from the statistical analyses which were carried out (as described in Part I) according to the usual method, for 5% points. Tables IIIA and B and IVA and B show the Analysis of Variance data and are given at the end of this paper.

(a) *Stocks—Months.* The changes from month to month in the carbohydrate content of bark and wood are essentially the same for all stocks, i.e. there is no significant difference in the seasonal cycles due to stock effect. With regard to leaves, the analysis shows a slight significant difference due to differences in the curves for sucrose and reducing sugars. In the sucrose cycle, stocks M.II and M.V show earlier maxima than the others, while in the reducing sugar curves the summer minima occur earlier for M.IX and M.XII than for M.II, M.V and M.VII. No great importance can be attached to these differences, owing to their irregularity, and also in view of the fact that the curves for starch and hemicellulose show no stock differences.

(b) *Stocks—Fractions.* The general level of carbohydrate content is the the same for all the stocks, i.e. the effect of stock on the concentration of the various fractions is not significant ; this applies to bark, wood and leaves.

(c) *Months—Fractions.* The cycles for the individual carbohydrate fractions differ in form for the various constituents, the change from month to month thus affecting each fraction differently. This interaction is therefore significant for bark, wood and leaves.

(d) *Stocks—Parts of Plant.* The ratio of the total content of all fractions in the bark, as compared with that in the wood, is approximately the same for all the stocks, i.e. there is no significant difference between them.

(e) *Fractions—Parts of Plant.* The ratio of the amount of individual fractions in bark as compared with the amount in wood, differs significantly for the various fractions. This interaction is obtained by summing the concentrations in all the stocks for each fraction separately, and comparing the ratio bark/wood content. Thus, the ratio of the starch content in bark to that in wood is significantly different from the bark/wood ratio for sugar content.

(f) *Months—Parts of Plant.* To obtain this interaction the sum of all the fractions for all stocks was taken and compared statistically for bark and wood. When the results for the twelve months are considered, the effect of change of season on the carbohydrate content is significantly different in the bark from that in the wood. The results for the seven months only do not show this significance, which suggests that the chief differences in the cycles for bark and wood occur during the dormant season.

TABLE IIIA.

Analysis of Variance of per cent. Carbohydrate, Alcohol Soluble Matter and Lignin Fractions in Bark and Wood Portions of Shoots.

(*Stocks M.II, M.V, M.VII, M.B. Results of twelve months' sampling.*)

Source of Variation.	Degrees of Freedom.	Sum of Squares.	Mean Square.	Z ½(log$_e$ Factor—log$_e$ Error).	5% Points.	Significance.
Between						
Means of Months ..	11	101·19	9·19	0·8791	0·2804	Yes.
,, Stocks ..	3	0·88	0·29	$\bar{1}$·1550	0·4787	No.
,, Fractions ..	7	109032·28	15576·04	4·6013	0·3706	Yes.
,, Parts of Plant	1	7·00	7·00	0·7474	0·6729	Yes.
Interactions						
Months—Stocks ..	33	6·66	0·20	$\bar{2}$·9697	0·2085	No.
,, Fractions ..	77	2923·21	37·96	1·5927	0·2085	Yes.
,, Parts of Plant	11	36·00	3·27	0·3668	0·2804	Yes.
Stocks—Fractions ..	21	7·46	0·35	$\bar{1}$·2495	0·2085	No.
,, Parts of Plant	3	0·20	0·06	$\bar{2}$·3677	0·4787	No.
Parts of Plant—Fractions	7	21147·69	3021·09	3·2811	0·3706	Yes.
Remainder	593	931·06	1·57	—	—	—
Total	767	134193·63				

TABLE IIIB.

(*Stocks M.II, M.V, M.VII, M.B and M.IX. Results of seven months' sampling.*)

Source of Variation.	Degrees of Freedom.	Sum of Squares.	Mean Square.	Z ½(log$_e$ Factor—log$_e$ Error).	5% Points.	Significance.
Between						
Means of Months ..	6	45·21	7·53	0·6938	0·3706	Yes.
,, Stocks ..	4	3·08	0·77	$\bar{1}$·5536	0·4319	No.
,, Fractions ..	7	78487·81	11212·54	4·3466	0·3706	Yes.
,, Parts of Plant	1	0·02	0·02	$\bar{3}$·7283	0·6729	No.
Interactions						
Months—Stocks ..	24	10·34	0·43	$\bar{1}$·2623	0·2085	No.
,, Fractions ..	42	3091·87	73·61	1·8337	0·2085	Yes.
,, Parts of Plant	6	18·10	3·01	0·2303	0·3706	No.
Stocks—Fractions ..	28	15·47	0·55	$\bar{1}$·3854	0·2085	No.
,, Parts of Plant	4	1·20	0·30	$\bar{1}$·0823	0·4319	No.
Parts of Plant—Fraction	7	13656·04	1950·86	3·4724	0·3706	Yes.
Remainder	430	809·20	1·88	—	—	—
Total	559	96138·34				

TABLE IVA.

Analysis of Variance of per cent. Carbohydrate, Alcohol Soluble Matter, Cellulose and Lignin Fractions in Leaves.

(Stocks M.II, M.V, M.VII, M.B. Results of six months' sampling.)

Source of Variation.	Degrees of Freedom.	Sum of Squares.	Mean Square.	Z ½(loge Factor—loge Error).	5% Points.	Significance.
Between						
Means of Months ..	5	40·41	8·08	1·2837	0·3974	Yes.
,, Stocks ..	3	1·25	0·41	$\bar{1}$·7932	0·4787	No.
,, Fractions ..	5	37373·81	7474·76	4·6986	0·3974	Yes.
Interactions						
Months—Stocks ..	15	28·52	1·90	0·5600	0·2804	Yes.
,, Fractions ..	25	102·47	4·09	0·9433	0·2085	Yes.
Stocks—Fractions ..	15	12·31	0·82	0·1398	0·2804	No.
Remainder	75	46·81	0·62	—	—	—
Total	143	57605·58				

TABLE IVB.

(Stocks M.II, M.V, M.VII, M.B and M.IX. Results of five months' sampling.)

Source of Variation.	Degrees of Freedom.	Sum of Squares.	Mean Square.	Z ½(loge Factor—loge Error).	5% Points.	Significance.
Between						
Means of Months ..	4	22·94	5·73	1·1718	0·4319	Yes.
,, Stocks ..	4	5·99	1·49	0·4983	0·4319	No.
,, Fractions ..	5	38355·10	7671·02	4·7715	0·3974	Yes.
Interactions						
Months—Stocks ..	16	16·26	1·01	0·3039	0·2804	Yes.
,, Fractions ..	20	51·11	2·55	0·7670	0·2085	Yes.
Stocks—Fractions ..	20	12·74	0·63	0·0679	0·2085	No.
Remainder	80	44·61	0·55	—	—	—
Total	149	38508·75				

CONCLUSIONS.

1. The seasonal cycles obtained for the Lane's Prince Albert trees on the five rootstocks agree in their essential features with those found in Newton Wonder trees under two cultural systems, both with regard to form of cycle and order of concentration of substances estimated.

2. It is apparent from the graphs and also from the statistical analysis that there are no significant differences between the seasonal cycles of carbohydrate fractions and of lignin in the terminal shoots of these trees on rootstocks M.II, M.V, M.VII, M.IX and M.B.

A tendency was observed, however, in trees on stock M.IX to accumulate starch and hemicellulose at a consistently higher rate during the summer and autumn, than in trees on the other stocks. This difference, though regular, was not statistically significant.

3. From these results and from a comparison with those for the Lane's and Newton trees it would thus appear that seasonal cycles of carbohydrate and lignin are unaffected by rootstocks in mature trees, and that different scion varieties may have similar cycles.

In the rootstocks, since these had effected significant pomological differences on the Lane's trees, e.g. in growth, it may be concluded that such differences are not correlated with the carbohydrate status in the terminal shoots.

4. The statistical analysis leads to the following further conclusions:—

(1) Total concentration of the respective constituents estimated is similar for the trees on all the rootstocks.

(2) The proportions of the constituents present in bark, as compared to the amounts in wood, are similar for all stocks.

5. The analysis also confirms the following points observed in the work on Newton Wonder, but not then statistically treated:—

(1) The various carbohydrate constituents and lignin have cycles which differ significantly from each other.

(2) The proportions of these fractions in bark as compared with the amounts in the wood, i.e. ratio bark content/wood content, differ significantly from one another.

(3) The seasonal cycles in bark are significantly different from those in the wood.

SUMMARY.

1. The seasonal cycles of carbohydrate constituents and of lignin in the terminal shoots of apple trees (var. Lane's Prince Albert) grafted on Malling stocks M.II, M.V, M.VII, M.IX and M.B, have been determined in the bark, wood and leaves, with the objects (1) of discovering the effect of the various stocks on the composition of the shoots, and (2) of comparing the results with previous work by the writer on seasonal carbohydrate cycles.

2. Sampling methods were similar to those described in the previous paper.

3. Chemical methods were also similar, with the exception of a revised method for starch which avoided the acid hydrolysis previously used after enzyme digestion. Total alcohol soluble matter, reducing sugars, sucrose, total sugars, starch, hemicellulose, cellulose and lignin were determined.

4. The results showed that there was great uniformity in the cycles of all the fractions for all the stocks. Starch and hemicellulose tend to accumulate from the end of June onwards more rapidly in M.IX than in the other four stocks, leading to a higher winter content.

5. The cycles showed the same characteristic features as those described for Newton Wonder on M.II in the earlier work, both in form of cycle and order of content of substances in the tissues.

LITERATURE REFERENCES.

(1) *Bish, E. J. B.* The determination of small quantities of starch in vegetable tissue. Biochem. Journ., 1929, **23**, 31.

(2) *Colby, H. L.* Stock-Scion chemistry and the fruiting relationships in apple trees. Plant Physiol., 1935, **10**, 483.

(3) *Denny, F. E.* Improvements in the methods of determining starch in plant tissue. Contrib. Boyce Thompson Inst., 1934, **6**, 129.

(4) *Gardner, F. E.* Composition and growth initiation of dormant Bartlett pear shoots as influenced by temperature. Plant Physiol., 1929, **4**, 405.

(5) *Niemann, C. G., Roberts, R. H.* and *Link, K. P.* Isolation and determination of starch in plant tissue. Plant Physiol., 1935, **10**, 579.

(6) *Shriner, R. L.* Starch determination in plant tissues. Plant Physiol., 1932, **7**, 541.

(7) *Sullivan, J. T.* Estimation of starch in woody plants. Abstracts Amer. Soc. Plant Physiol., Pittsburg Meeting, 1934, 11.

(8) *Smyth, E. S.* The seasonal cycles of nitrogenous and carbohydrate materials in fruit trees. Journ. Pom. & Hort. Sci., 1934, **12**, 249.

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STUDIES ON FRUIT BUD FORMATION IN DECIDUOUS FRUIT TREES IN SOUTH AFRICA

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I. GROWTH AND FRUIT BUD DIFFERENTIATION IN SOME VARIETIES OF DECIDUOUS FRUITS

A KNOWLEDGE of the internal development of the fruit bud, prior to its expansion, and of its relationship to shoot growth is of value to the horticulturist. Although fruit bud differentiation has been studied by research workers such as Albert (1), Askenasy (2) and Elsmann (8) in Germany ; Ball (3) and Gibbs and Swarbrick (9) in England ; Barnard and Read (4) in Australia ; Bijhouwer (5), Luyten (12), Luyten and de Vries (13) and Versluys (17) in Holland ; Johansson (11) in Sweden ; Malan (14) in South Africa ; Watanabe and Yasaka (18) in Manchuria ; and Bradford (6), Drinkard (7), Goff (10), Rasmussen (15) and Tufts and Morrow (16) in America, little work has been done on this problem in South Africa.

The present report outlines an attempt by the author to obtain local information on the time of fruit bud formation, and its relationship to shoot growth.

A. Bud Studies.

The aim of these bud studies was to determine the time of differentiation and to ascertain the internal development of buds of certain varieties of apples, pears, peaches, plums and apricots, prior to the resting period. All the material used was derived from the variety orchards of the University experimental farm at Stellenbosch, the trees being in full bearing. These trees received normal cultural treatment, were planted on an alluvial loam, and were not irrigated during the summer months.

For each variety a sample was taken from two adjacent trees, outwardly potential fruit buds being selected in all cases. During the first season the buds were collected at fortnightly intervals from 12/11/34 to 20/2/35, with a final collection on 14/3/35 ; while in the second season they were collected fortnightly from 14/11/35 to 12/3/36, with a final sample of Bon Chrêtien pear buds on 4/4/36. On sampling, the buds were placed in 80 per cent. alcohol, in which they were stored until June, when they were examined.

In all cases the buds were examined by the "dissection method" described by Bijhouwer (5), Luyten (12), Luyten and de Vries (13), Versluys (17), Malan

(14) and Barnard and Read (4). This method is quicker than the microtome-sectioning one, although the latter is essential for a study of the winter development of the ovary, pollen grains, etc.

The buds were dissected at a magnification ranging from 50-100 times, while artificial light was projected straight on to the object examined. The bud scales were removed under the microscope with a lancet-needle, while the flowers were dissected with scalpels made by inserting fragments of razor blades in sealing wax on the ends of pieces of glass tubing. In most cases the internal parts of the buds were coloured light brown, but when additional staining was necessary, the buds were soaked in a strong solution of iodine in potassium iodide after removal of the outer scales.

On dissection, the buds were classified into seven stages of internal development, and a brief summary of these stages will now be given. For a fuller description see Barnard and Read (4) for pome fruits and Luyten (12) for stone fruits. During the course of this work 2,900 buds were dissected and examined microscopically.

Stages of Internal Development of Fruit Buds.

Stage I: Prior to differentiation; axis of buds is short and growing point flat and inconspicuous.

Stage II: Differentiation takes place; growing point broad and rounded.

Stage III: Axillary primordia differentiate into bractlets and flower primordia proper in the buds of pome fruits, while in those of stone fruits flower primordia arise in the axil of each bract.

Stage IV: Formation of sepals in terminal flower.

Stage V: Petal initials produced in terminal flower.

Stage VI: Stamen initials formed in three whorls.

Stage VII: Carpels appear as five prominences from the base of the receptacle. In stone fruits the carpel arises from the base of the receptacle and rapidly grows into a dome-shaped structure.

The buds were not studied further than Stage VII.

B. Growth Studies.

Concurrently with the selection of buds for examination, the growth in length of shoots was studied. A number of shoots growing from the terminal section of laterals were tagged, and their growth increases were recorded in centimetres. Diameter increases of two-year-old wood were also measured, the readings being taken with calipers 2 cm. below the base of each of the shoots the growth in length of which was recorded. These diameter readings were

continued until growth in length had ceased in 1935, but were not continued during the 1935-36 season.

RESULTS.

(a) STONE FRUITS.

When sampling buds of Japanese plums and the Early Cape apricot, material was selected from current shoots as well as from spurs* originating from two-year-old wood. With Elberta and Peregrine peach, all material

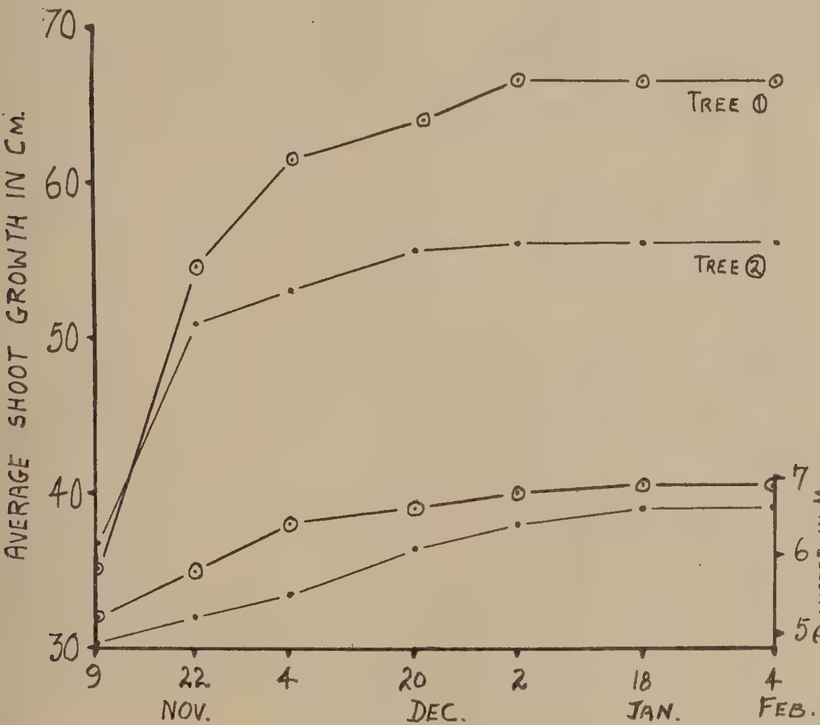

FIG. 1.

Shoot growth and diameter increase of two-year-old wood of the Santa Rosa plum.

selected was of the triple bud type, situated on shoots where the central bud was a leaf bud and the two outer ones were flower buds.

Table I presents the results obtained from buds situated on spurs of Japanese plums and of Early Cape apricot, and for buds on shoots of Elberta and Peregrine peach. It shows that for the varieties of stone fruits studied during the 1934-35 and 1935-36 seasons, the critical period of fruit bud initiation lay between the beginning of January and the middle of February.

* This type of growth is referred to as fruit shoots by some authors.

TABLE I.

Showing the period when the majority of the buds of each variety reached the various stages of internal development. (The dates in brackets indicate when buds were first noticed in each stage.)

Variety.	Stage II.	Stage III.	Stage IV.	Stage V.	Stage VI.	Stage VII.
Japanese Plums : Santa Rosa ..	1st week in Jan. (24/12/34)	2nd and 3rd week in Jan. (4/1/35)	Late Jan and 1st week in Feb. (4/1/35)	Early Feb. (19/1/35)	Late Feb. (19/1/35)	1st and 2nd week in Mar. (4/2/35)
Gaviota ..	1st week in Feb. (4/2/35)	3rd and 4th week in Feb. (4/2/35)	1st and 2nd week in Mar. (14/3/35)	(14/3/35)	No further development noted up to 14/3/35	
Kelsey ..	1st, 2nd and 3rd week in Feb. (4/2/35)	1st and 2nd week in Mar. (20/2/35)	No further development noted up to 14/3/35			
Apricot : Early Cape ..	1st and 2nd week in Feb. (4/2/35)	Late Feb. (20/2/35)	Mid. Mar. (14/3/35)	Mid. Mar. (14/3/35)	Mid. Mar. (14/3/35)	Late Mar. (14/3/35)
Peaches : Elberta ..	Mid. Jan. (19/1/35)	(19/1/35)	(19/1/35)	(20/2/35)	(20/2/35)	Early Mar. (20/2/35)
Peregrine 1934-1935	3rd and 4th week in Jan. (19/1/35)	Most buds in stage VII by mid. Mar. (19/1/35)	(20/2/35)	(20/2/35)	(20/2/35)	(20/2/35)
Peregrine 1935-1936	3rd and 4th week in Jan. (16/1/36)	Most buds in stage VII by end Feb. (30/1/36)	(30/1/36)	(30/1/36)	(30/1/36)	(27/2/36)

As regards the development of fruit buds on current shoots of the Japanese plums and the Early Cape apricot, it was found that differentiation began one to three weeks later than in buds situated on spurs. Throughout the period of examination a more rapid rate of internal development was shown by spur buds than by buds on shoots. Similar results were obtained by Barnard and Read (4) with the Satsuma plum in Australia.

In dealing with the growth studies, Fig. 1, showing the growth curves of the two Santa Rosa plum trees, will illustrate the results obtained. From this it is seen that fruit bud differentiation began in this variety shortly after cessation of growth. Similar results were obtained with the other varieties of stone fruits and also on trees of the same variety. Here, again, the results confirm the findings of Barnard and Read (4), except that for peaches they found that differentiation started just prior to cessation of growth in length.

The diameter increase of two-year-old wood of Japanese plums ceased at the same time as growth in length, while in the apricot and the peaches no such tendencies were shown.

(b) POME FRUITS.

A randomized sample of pome fruit buds situated on the terminals of young spurs was collected from two trees of each variety. The results obtained are given in Table II, and on comparing the results given for pome fruits in this Table, it appears that for most varieties the critical period for fruit bud initiation was December.

With the exception of the Kieffer pear, it was found that in the varieties studied differentiation started shortly after shoot growth had ceased. The Kieffer pear, however, started to form blossom buds shortly before cessation of shoot growth.

In all varieties diameter increase of two-year-old wood continued after shoot growth had ended.

DISCUSSION OF RESULTS.

As has been pointed out by Ball (3), "a rigid date cannot be given as the time for the change of the vegetative into the fruit bud. The factors causing this variation are variety, season, position of the bud on the tree and cultural treatment." At Stellenbosch, climatic conditions fluctuate so widely and influence tree behaviour to such an extent that a definite period for fruit bud differentiation cannot be determined after only two seasons' work.

However, taking the results as a whole, it appears that under local conditions, fruit bud differentiation starts shortly after cessation of growth in length of the shoot. As this confirms the work of Barnard and Read (4), it offers an index for determining the approximate time when fruit bud formation begins.

TABLE II.

Showing the period when the majority of the buds of each variety reached the various stages of internal development. (The dates in brackets indicate when buds were first noticed in each stage.)

Variety.	Stage II.	Stage III.	Stage IV.	Stage V.	Stage VI.	Stage VII.
Apples : Cliff's Seedling	4th week in Dec. and 1st week in Jan. (24/12/34)	Early Jan. (19/1/35)	Late Jan. (19/1/35)	Early Feb. (19/1/35)	Late Feb. (19/1/35)	(14/3/35)
Jonathan	4th week in Dec. and 1st week in Jan. (24/12/34)	Early Jan. (19/1/35)	Late Jan. (19/1/35)	Early Feb. (4/2/35)	Late Feb. (20/2/35)	(14/3/35)
White Winter Pearmain	Late Jan. (19/1/35)	Early Feb. (19/1/35)	Late Feb. (4/2/35)	Early Mar. (20/2/35)	(20/2/35)	Not reached by (14/3/35)
Pears : Beurré Bosc	Late Dec. (5/12/34)	Early Jan. (4/1/35)	Late Jan. (4/1/35)	Early Feb. (19/1/35)	Late Feb. (4/2/35)	Early Mar. (14/3/35)
Beurré Hardy	Early Jan. (4/1/35)	Late Jan. (19/1/35)	Early Feb. (19/1/35)	Late Feb. (20/2/35)	Early Mar. (20/2/35)	Not reached by (14/3/35)
Glou Morceau	Late Dec. (24/12/34)	Early Jan. (24/12/34)	Late Jan. (4/1/35)	Early Feb. (19/1/35)	Late Feb. (4/2/35)	Early Mar. (14/3/35)
Kieffer	Late Nov. (24/11/24)	Early Dec. (5/12/34)	Late Dec. (24/12/34)	Early Jan. (4/1/35)	Late Jan. (19/1/35)	Early Mar. (19/1/35)
Packham's Triumph	Early Jan. (4/1/35)	Late Jan. (19/1/35)	Early Feb. (19/1/35)	Late Feb. (4/2/35)	Late Feb. (4/2/35)	Early Mar. (14/3/35)
Bon Chrêtien (1934-1935)	Late Dec. (24/12/34)	Early Jan. (4/1/35)	Late Jan. (4/1/35)	Early Feb. (19/1/35)	Late Feb. (19/1/35)	Early Mar. (14/3/35)
Bon Chrêtien (1935-1936)	Late Dec. (28/12/35)	Early Jan. (28/12/35)	Early Jan. (28/12/35)	Late Jan. (16/1/36)	Early Feb. (16/1/36)	Late Feb. (12/2/36)

In conclusion, a comparison of the results obtained by workers on this problem in the Southern Hemisphere is shown in Table III. The data indicate

TABLE III.

Tabulation of dates of fruit bud differentiation.

Variety.	Locality.	Differentiation first noted.	Research Worker.
Plums (spur buds) :			
Grand Duke	Templestowe, Victoria, Australia	26/12/30	Barnard & Read (4)
Satsuma ..	Do. do. ..	6/ 2/31	do.
*Prune d'Agen	Shepparton, Victoria, Australia	22/12/31	do.
Santa Rosa ..	Stellenbosch, South Africa ..	24/12/34	Micklem.
Gaviota ..	Do. do. ..	4/ 2/35	do.
Kelsey ..	Do. do. ..	4/ 2/35	do.
Apricots (spur buds) :			
Moorpark ..	Templestowe, Victoria, Australia	30/ 1/31	Barnard & Read (4)
Moorpark ..	Shepparton, Victoria, Australia	12/ 1/31	do.
Early Cape ..	Stellenbosch, South Africa ..	4/ 2/35	Micklem.
Peaches :			
Anzac ..	Templestowe, Victoria, Australia	9/ 1/31	Barnard & Read (4)
Pullar's Cling	Shepparton, Victoria, Australia	12/ 1/31	do.
Elberta ..	Stellenbosch, South Africa ..	19/ 1/35	Micklem.
Peregrine ..	Do. do. ..	19/ 1/35	do.
Peregrine ..	Do. do. ..	16/ 1/36	do.
Apples (spur buds) :			
Yates ..	Templestowe, Victoria, Australia	12/12/30	Barnard & Read (4)
Jonathan ..	Do. do. ..	12/12/30	do.
Rome Beauty	Do. do. ..	2/ 1/31	do.
Cleopatra ..	Harcourt, Victoria, Australia ..	4/12/30	do.
Dunn's Seedling ..	Do. do. ..	31/12/30	do.
White Winter Pearmain ..	Stellenbosch, South Africa ..	1/ 1/33	Malan (13).
Ohenimuri ..	Do. do. ..	17/ 1/33	do.
Wemmershoek	Do. do. ..	31/ 1/33	do.
Cliff's Seedling	Do. do. ..	24/12/34	Micklem.
Jonathan ..	Do. do. ..	24/12/34	do.
White Winter Pearmain	Do. do. ..	19/ 1/35	do.
Pears (spur buds) :			
Bon Chrêtien	Shepparton and Templestowe, Victoria, Australia	1st week in Dec., 1930	Barnard & Read (4)
Bon Chrêtien	Stellenbosch, South Africa ..	24/12/34	Micklem.
Bon Chrêtien	Do. do. ..	28/12/35	do.
Beurré Bosc	Do. do. ..	5/12/34	do.
Beurré Hardy	Do. do. ..	4/ 1/35	do.
Glou Morceau	Do. do. ..	24/12/34	do.
Kieffer ..	Do. do. ..	24/11/34	do.
Packham's Triumph ..	Do. do. ..	4/ 1/35	do.

* Axillary buds on current season's growth.

that the results obtained in the present study are comparable with the findings of other workers.

ACKNOWLEDGMENTS.

The author wishes to thank Prof. O. S. H. Reinecke, under whose guidance this work was carried out; Mr. M. W. Black, who collected most of the material for the first season's work; and Mr. P. E. Kriel, who helped with the recording in 1935.

SUMMARY.

Buds from three varieties of Japanese plum, one of apricot, two of peach, three of apple and six of pear were dissected, to determine when differentiation started and to trace the internal development of fruit buds prior to the resting period. The results obtained are tabulated for seven defined stages of internal development of the buds.

Measurements of shoot growth and of diameter increase of two-year-old wood were made on all the varieties studied. It was found that, with the exception of the Kieffer pear, differentiation started shortly after cessation of shoot growth. In the Kieffer pear differentiation began just prior to shoot growth cessation.

The results of other workers in the Southern Hemisphere for dates of fruit bud differentiation are tabulated for purposes of comparison.

REFERENCES.

(1) *Albert*, —. Entwicklungsgeschichte der Knospen einiger Laubhölzer. Forstnaturwiss, Z. 1894.

(2) *Askenasy, E.* Über die jährliche Periode der Knospen. Bot. Ztg., 1894, **35**, 793.

(3) *Ball, E.* The time of differentiation and the subsequent development of the blossom bud of the plum. Journ. Pom. & Hort. Sci., 1927, **6**, 198.

(4) *Barnard, C.* and *Read, F. M.* Studies of growth and fruit bud formation. Journ. Dept. Agric., Vict., Aust., 1933, **31**, 37.

(5) *Bijhouwer, J.* De periodiciteit van de Knopontwikkeling bij den Appel. Med. LdbHoogesch. Wageningen. 1924, Deel XXVII, No. 9.

(6) *Bradford, F. C.* Fruit bud development of the apple. Ore. Agric. Col. Expt. Sta., 1915, Bul. 129.

(7) *Drinkard, A. W.* Fruit bud formation and development. Ann. Rep. Virg. Polytech. Inst. Agric. Expt. Sta., 1909-1910.

(8) *Elsmann, E.* Über die Periodicität der Blütenentwicklung bei den Obstgewächsen. Landw. Jahrb., 1925, **62**, 539.

(9) *Gibbs, M. A.* and *Swarbrick, T.* Time of differentiation of the flower bud of the apple. Journ. Pom. & Hort. Sci., 1930, **8**, 61.

(10) *Goff, E. S.* The origin and early development of the flowers in the cherry, plum, apple and pear. Rep. Wisc. Expt. Sta., 1899, 16; 1900, 17; 1901, 18.

(11) *Johansson, E.* Undersökningar över Blomknopps-anläggningen hos frukttträd. Sveriges Pomologiska Förenings Årsskrift, 1930, **31**, 1.

(12) *Luyten, I.* De periodiciteit van de knopontwikkeling bij den pruim. Med. LdbHoogesch. Wageningen., 1921, Deel XVIII.

(13) *Luyten, I.* and *de Vries, E.* De periodiciteit van de Knopontwikkeling bij den Peer. Uitgave koninkl. Akad. van Wetensch. Amsterdam, 1926.

(14) *Malan, P. F.* Die periodiciteit van die vroeëre stadia in die vorming van bloome by bladwisselende vrugtebome. Unpublished thesis for M.Sc. degree, Stellenbosch, 1933.

(15) *Rasmussen, E. J.* The period of blossom bud differentiation in the Baldwin and McIntosh apples. New Hamp. Agric. Expt. Sta., 1930, Sci. Contrib. 26.

(16) *Tufts, W. P.* and *Morrow, E. B.* Fruit bud differentiation in deciduous fruits. Hilgardia, 1925, **1**, 1.

(17) *Versluys, M. C.* De periodiciteit van de knopontwikkeling bij den kers. Med. LdbHoogesch. Wageningen., 1921, Deel XVIII.

(18) *Watanabe, R.* and *Yasaka, T.* Studies on the time of fruit bud formation and development of apples in Manchuria. South Manchurian Railway Co., Agric. Expt. Sta., 1930, Res. Bull. 1.

II. THE EFFECT OF PRUNING AND SHADING ON FRUIT BUD DIFFERENTIATION AND GROWTH IN THE PEREGRINE PEACH

The object of this study was to determine the effect of pruning and shading treatments on (1) the time of fruit bud differentiation, and (2) shoot growth and leafing out of the Peregrine peach.

The methods described in Part I were employed in collecting and examining fruit buds and in recording growth during the 1934-35 season. Growth records during the second year were more detailed, the additional refinements introduced being described in the following section of this paper.

Effect of Pruning on Growth and Fruit Bud Formation.

Season 1934-35. During the first season the effect of pruning on growth and bud differentiation was studied. Four uniform nine-year-old Peregrine peach trees were selected, all of which were planted on the 20 ft. square system, and were on peach seedling roots. The soil was an alluvial loam about five feet deep. The relative positions of these trees, and of a fifth and sixth tree, used for shading experiments, during the 1935-36 season are shown in Fig. 1.

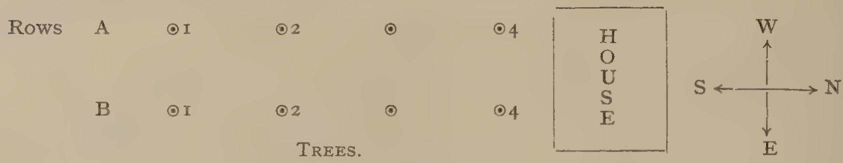

Fig. 1.
Plan of Peregrine Trees.

The trees in row A were all long-pruned while those in row B were short-pruned. Long-pruning consisted of thinning out fruiting wood, leaving enough wood to ensure normal cropping. In short-pruning, less thinning out was done and all shoots (fruiting wood) were moderately headed back. Apart from the different pruning methods used, all these trees received similar cultural treatment, while no irrigation water was applied during the summer.

The results obtained during the summer of 1935, showing the relation of the time of fruit bud differentiation to the pruning practices described above, are presented in Table I, and from them it will be seen that for both long- and short-pruned trees, differentiation had started by January 19th, while by March 14th most buds had reached Stage VII. Thus, under the conditions of the experiment, the different pruning treatments had no effect on the time of differentiation.

Table I.

Showing the period when the majority of the buds reached the various stages of of internal development. (Dates in brackets indicate when buds first reached these stages.)

Variety.	Stage II.	Stage III.	Stage IV.	Stage V.	Stage VI.	Stage VII.
Peregrine Peach Buds from trees A1 and A2 (long-pruned)	Late Jan. (19/1/35)	Early Feb. (19/1/35)	Mid. Feb. (20/2/35)	Late Feb. (20/2/35)	Early Mar. (20/2/35)	Early Mar. (20/2/35)
Peregrine Peach Buds from trees B1 and B2 (short-pruned)	Late Jan. (19/1/35)	Early Feb. (19/1/35)	Mid. Feb. (19/1/35)	Late Feb. (20/2/35)	Early Mar. (20/2/35)	Early Mar. (20/2/35)

Individual growth curves showing the average shoot growth for these four trees are shown in Fig. 2. From these it is evident that the average length of shoot growth on the short-pruned trees (B_1 and B_2) was greater than that of the long-pruned trees (A_1 and A_2), while shoot growth in both cases had stopped by January 18th. Thus, shoot growth ceased just prior to the first evidence of differentiation (see Table I).

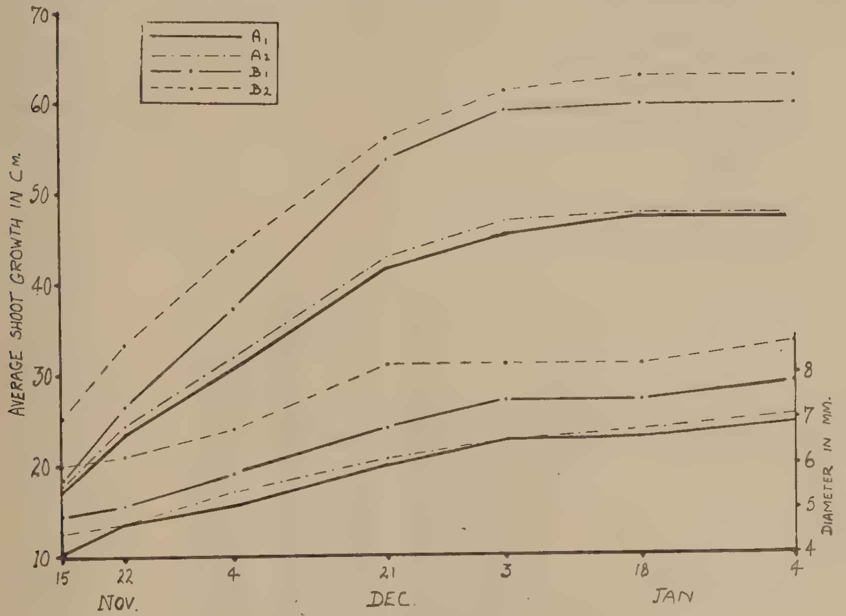

FIG. 2.

Shoot growth and diameter increase of two-year-old wood of the Peregrine Peach (1934-35).

The diameter increase of two-year-old wood continued rapidly after the cessation of shoot growth. This is in accordance with the findings of Barnard and Read (3), who state that for the Anzac and the Pullar's peach, diameter increase of two-year-old wood starts early in the growing season and extends almost up to the time of defoliation.

EFFECT OF PRUNING AND SHADING ON FRUIT BUD DIFFERENTIATION.

Season 1935-36. During this season buds were sampled from trees A_2, A_4, B_2 and B_4 (see Fig. 1). Trees in row A were long-pruned while those in row B were short-pruned, while in addition trees A_4 and B_4 were partially shaded throughout the year by a house on the northern side.

Approximately twenty buds were selected from these trees at fortnightly intervals, from November 14th, 1935, to March 12th, 1936. All buds collected

were of a strong triple bud formation, situated on the central portion of shoots of medium vigour. With such buds, the two lateral fruit buds usually showed the same stage of internal development, though this was not always so. During dissection and subsequent classification of bud development it was noticed that the growing point was rounded and clearly visible from November onwards. Two to three weeks before differentiation started, the scale initials were split off in rapid succession, while shortly after this occurred the growing point swelled out, and was raised up on its axis. This stage, first noticed on

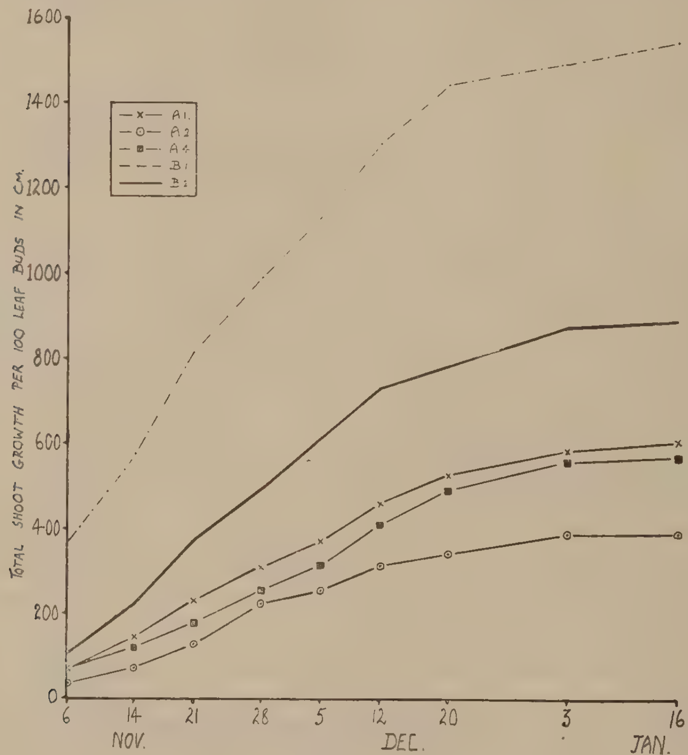

FIG. 3.
Shoot growth per 100 leaf buds.

January 16th for all four trees, was taken as the first indication that fruit bud differentiation had begun. After this date the buds on all the trees showed a steady development up to February 27th, when the majority of them had reached Stage VII (carpel rising as a protuberance from the base of the receptacle). By March 12th all buds examined had reached Stage VII. These observations showed that under the conditions at Stellenbosch during the 1935-36 season, shading combined with either short- or long-pruning, had no

effect on the time or rate of differentiation in the trees studied. Here, again, the different pruning treatments did not influence the time of differentiation.

EFFECT OF PRUNING AND SHADING ON GROWTH.

(1) *Shoot growth.* The records on tree growth made in 1935-36 were of a more detailed nature than those taken the previous season. They were taken on trees A_1, A_2, A_4, B_1 and B_2. Tree A_4 was partially shaded by a house throughout the year, while B_1 was artificially shaded from May to September 1935. Eight comparable two-year-old branches* were selected on each tree on November 6th, 1935. These branches were chosen as representing the normal growth habits of the trees, and were spaced at regular intervals around

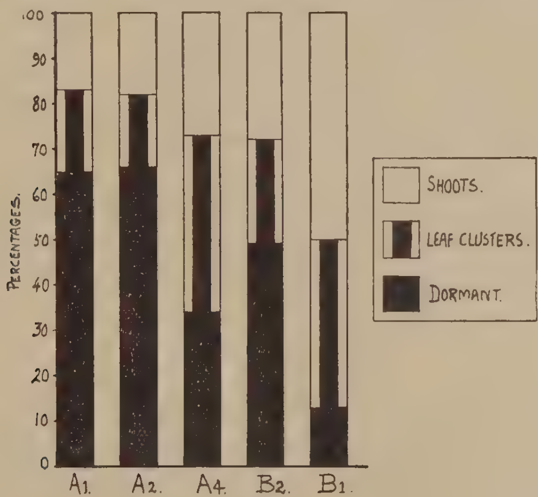

FIG. 4.
The effect of shading on the leaf bud development of the Peregrine Peach.

each tree. On these branches the leaf bud, shoot and leaf cluster development was recorded, while after cessation of shoot growth the final development of secondary, or side-shoot growth, was measured.

The results presented in Fig. 3 illustrate the development of shoots, taking total shoot growth per 100 leaf buds as the unit at each examination. This type of record gives a truer picture of tree performance than the average length of shoot growth. The apparent effect of artificial shading during the winter months is shown in this figure. Tree B_1 (artificially shaded on the northern side, May-September 1935) developed 1,545 cm. of shoot growth per 100 leaf buds, regardless of secondary growth, as compared with 890 cm. in B_2 (not

* By two-year-old branches is implied fruiting wood entering its second growth.

shaded). Tree A_4, which was also shaded, does not show this striking increase in shoot growth. The reason for this is probably the fact that this tree suffers from crown-gall (*Bacterium tumefaciens*). This malady was noticed only in November 1936.

In all cases, shoot growth had ceased by November 16th, just prior to differentiation.

(2) *Leaf bud development.* The effect of shading on leaf bud development of the Peregrine peach is shown in Fig. 4. These results indicate that in the two shaded trees A_4 and B_1, more shoots and leaf clusters were developed per 100 leaf buds than in the non-shaded trees. The figures showing the effect of shading have been analysed statistically, and the results, together with those shown in Fig. 4, are assembled in Table II. They show that there is no significant

TABLE II.

The effect of shading on leaf bud development of the Peregrine Peach.

Tree.	Per 8 Shoots.				
	Total No. of Leaf Buds.	No. of Shoots.	No. of Leaf Clusters.	No. of Dormant Buds.	Mean percentage and Standard Error of Dormant Buds.
A1	217	37	39	141	64·97 ±2·72
A2	180	33	29	118	65·55 ±4·66
A4	201	54	79	68	33·83 ±4·67
B1	98	49	36	13	13·26 ±5·72
B2	119	33	28	58	48·73 ±6·71

difference in leaf bud development between trees A_1 and A_2 (long-pruned, non-shaded), while there is a highly significant difference between these two and tree A_4 (long-pruned, partially shaded throughout the year). Comparing tree B_2 (short-pruned, non-shaded) and tree B_1 (short-pruned, artificially shaded May-September 1935), highly significant differences are also shown. Thus, shading appears to have stimulated the development of leaf buds in both long- and short-pruned trees.

TABLE III.

Showing the amount of secondary growth per 100 leaf buds.

Tree.	Secondary Growth per 100 Leaf Buds.
A1	87·5 cm.
A2	24·4 cm.
A4	0
B1	441·8 cm.
B2	212·1 cm.

(3) *Secondary growth.* A summary of measurements of secondary growth is given in Table III, from which it is seen that tree B_1 (artificially shaded) developed twice as much secondary growth as B_2 (not shaded). A_4 (partially shaded) developed no secondary growth, but, as has been explained, this was thought to be due to a pathological condition of the tree.

At this stage no explanation is advanced as to what are the various physical and micro-climatic effects of shading on the Peregrine peach, as this problem is still under investigation.

SUMMARY.

During the 1934-35 season, it was found that neither short- nor long-pruning influenced the time of fruit bud differentiation of the Peregrine peach. The average shoot growth on short-pruned trees was greater than on long-pruned ones, while shoot growth in both ceased at the same time. The diameter increase of two-year-old wood continued after cessation of shoot growth.

In the following season (1935-36), short- and long-pruning alone, or combined with partial shading throughout the year, had no effect on the time of differentiation.

Artificial shading from May to September 1935 caused substantial increase in shoot growth.

Shading of both long- and short-pruned trees stimulated leaf bud development.

REFERENCES.

(1) *Auchter, E. C., Schrader, A. L., Lagasse, F. S.* and *Aldrich, W. W.* The effect of shade on growth, fruit bud formation and chemical composition of apple trees. Proc. Amer. Soc. Hort. Sci., 1926, **23**, 368.

(2) *Bailey, J. S.* Autumn development of peach fruit buds. Proc. Amer. Soc. Hort. Sci., 1924, **21**, 30.

(3) *Barnard, C.* and *Read, F. M.* Studies of growth and fruit bud formation. Journ. Dept. Agric., Vict., Aust., 1933, **31**, 37.

(4) *Gourley, J. H.* The effect of shading some horticultural plants. Proc. Amer. Soc. Hort. Sci., 1920, **17**, 256.

(5) *Horsfall, F.* Some factors influencing peach bud differentiation. Proc. Amer. Soc. Hort. Sci., 1927, **24**, 210.

(6) *Kraybill, H. R.* Effects of shading on some horticultural plants. Proc. Amer. Soc. Hort. Sci., 1922, **19**, 9.

(7) *Marshall, R. E.* The fruiting habit of the peach as influenced by pruning practices. Agric. Expt. Sta. Mich. State Coll., 1931, Bull. 116.

(8) *Pickett, B. S.* The effect of certain soil treatments on the formation of fruit buds in peaches. Proc. Amer. Soc. Hort. Sci., 1921, **18**, 80.

(9) *Quaintance, A. L.* The development of the fruit buds of the peach. Ga. Agric. Expt. Sta., 1901, Rpt. 13.

(10) *Reinecke, O. S. H.* Deciduous fruit investigations. I. Environment and its influence on fruit production. U. of S.A. Dept. Agric. Sci., 1936, Bull. No. 154.

(11) *Wiggans, C. B.* The influence of certain environmental and cultural conditions on fruit-bud formation of pear and apricot. Journ. Agric. Res., 1925, **31**, 865.

III. SOME EFFECTS OF WINTER OIL SPRAYS ON FRUIT BUD FORMATION AND LEAF BUD DEVELOPMENT IN THE BON CHRÊTIEN PEAR

Mg.

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It has been shown by Mally (8) and Black (2) that various winter oil sprays have a beneficial effect on blossoming, fruit production and growth of deciduous fruit trees such as pears, apples and plums. The aim of the present study was to determine some of the effects of these sprays on fruit bud formation and leaf bud development in the Bon Chrêtien pear.

On August 29th, 1935, one half of each of four Bon Chrêtien pear trees on the University experimental farm at Stellenbosch were sprayed with dormant oil sprays by Black (2). The four trees were 20 years old, on *communis* seedling stocks, planted 20 ft. apart ; prior to this experiment they had received similar cultural treatment. Before spraying, a canvas sheet was drawn through the main branches of each tree and held in position, dividing it into two equal portions ; one half of the tree was then sprayed, the remaining half being left unsprayed. The author was kindly allowed to collect buds from these trees to determine the effect of the treatment on fruit bud differentiation. At fortnightly intervals from 14/11/35 to 4/4/36, five potential fruit buds situated on two-year-old wood were selected from each tree-half. These buds were stored, and examined, as described in Part I of this paper. In addition, the areas of the leaves produced by the spurs sampled were measured with a planimeter on three of the bud-collection dates. Concurrently with the bud collections, detailed growth records were taken on eight two-year-old branches on each tree-half.

A second experiment was conducted at Groot Drakenstein, where the effect of various winter oil sprays on fruit bud formation was tested.

Western Cape Province

(1) STELLENBOSCH RESULTS.

The relative positions of the Bon Chrêtien pear trees are shown in Fig. 1, as well as the different spray treatments applied.

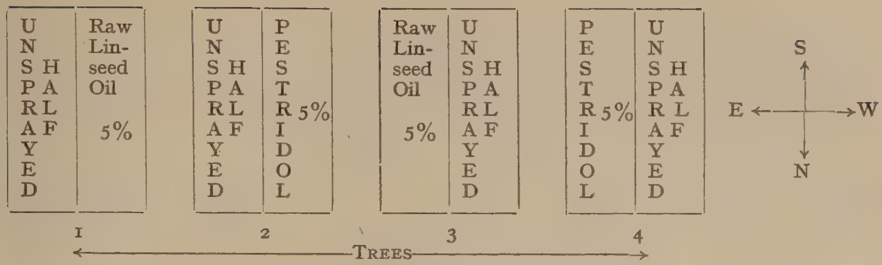

FIG. 1.

The treated tree-halves were sprayed on August 29th, 1935, with 5 per cent. oil emulsions, Pestridol* being a proprietary mineral oil (80 per cent. actual oil by weight) while linseed oil is a vegetable oil. Of the latter, a casein-ammonia emulsion was made. It was thought that by comparing treatment with control on each tree more accurate results would be obtained than by comparing treated and untreated separate trees, as the variable rootstock factor was thus eliminated.

EFFECT OF SPRAYS.

(a) *On fruit bud differentiation and shoot growth.* On examination of the buds collected from trees (1) and (3) (half sprayed with 5 per cent. raw linseed oil emulsion), the initial stage of differentiation in the buds on the sprayed tree-halves in both cases was first noticed on December 6th, 1935; while on the unsprayed halves this stage was first observed on December 28th, 1935. Considering these results in relation to the shoot growth curves of these trees during that season given by Black (2), it was evident that the first signs of differentiation practically coincided with the cessation of shoot growth.

In trees (2) and (4) (half sprayed with Pestridol), differentiation in the buds on the sprayed halves started between examinations made on December 6th and 20th, probably about the 12th, as by the 20th some of the buds had reached Stage IV. On unsprayed halves differentiation started between December 20th and 28th. Here again the results and the growth curves given by Black (2) show that differentiation and shoot growth cessation practically coincided. Thus, under the conditions of this experiment, these winter oil sprays advanced the initiation of fruit bud formation by 2-3 weeks. A possible explanation of this phenomenon is that shoot growth on the sprayed halves started earlier and ceased earlier than on the unsprayed halves (Black (2)).

(b) *On leaf bud development.* The effect of the two winter oil sprays on the development of leaf buds on these trees has been shown by Black (2). In Table I the results together with an analysis of the effect of these oils on leaf bud development are given.

* Kindly supplied by "African Explosives & Industries Ltd.," Somerset West.

TABLE I.

The effect of Pestridol and Raw Linseed Oil on leaf bud development in Bon Chrêtien Pear.

Tree-half.	Per 8 two-year-old Branches.					
	Treatment.	Total No. of Leaf Buds.	No. of Shoots.	No. of Spurs.	No. of Dormant Buds.	Mean percentage of Dormant Buds and Standard Error.
1	5% Raw Linseed Oil ..	102	23	42	37	36·27 ±*7·49*
1	Control (unsprayed)	113	12	7	94	83·18 ±*2·66*
3	5% Raw Linseed Oil ..	100	14	33	53	53·00 ±*4·49*
3	Control (unsprayed) ..	85	9	3	73	85·88 ±*6·43*
2	5% Pestridol ..	89	15	18	56	62·92 ±*7·11*
2	Control (unsprayed) ..	103	9	7	87	84·46 ±*2·76*
4	5% Pestridol ..	104	8	54	42	40·38 ±*7·76*
4	Control (unsprayed)	94	7	11	76	80·85 ±*8·48*

TABLE II.

The effect of Raw Linseed Oil and Pestridol on leaf development of spurs on two-year-old branches.

Treatment.	Date of of Examination.	Sprayed.		Unsprayed.	
		Average Leaf Area per 5 Spurs in sq. ins.	Average No. of Leaves per 5 Spurs.	Average Leaf Area per 5 Spurs in sq. ins.	Average No. of Leaves per 5 Spurs.
Tree 1, half sprayed with Raw Linseed Oil Emulsion	14/11/35 6/12/35 16/ 1/36	17·59 20·29 19·52	7·0 7·4 7·8	11·79 15·49 12·86	5·8 5·2 5·4
Tree 3, half sprayed with Raw Linseed Oil Emulsion	14/11/35 6/12/35 16/ 1/36	19·73 16·47 16·01	7·6 6·8 6·8	14·82 13·13 14·31	5·8 4·6 5·4
Average for trees 1 and 3 (Raw Linseed Oil)		18·26	7·23	13·73	5·36
Tree 2, half sprayed with Pestridol Emulsion ..	14/11/36	20·93	7·9	13·73	5·2
Tree 4, half sprayed with Pestridol Emulsion ..	14/11/36	14·64	7·4	13·37	6·6
Average for trees 2 and 4 (Pestridol) ..		17·78	7·65	13·55	5·90

These results clearly show that on the sprayed halves there was a significantly lower percentage of dormant leaf buds than on unsprayed (control) halves, while when comparing the four unsprayed halves, no significant differences could be detected. Furthermore, these results show that the winter oils promoted an increased development of fruit spurs, while their effect on total shoot growth is adequately shown in Fig. 2.

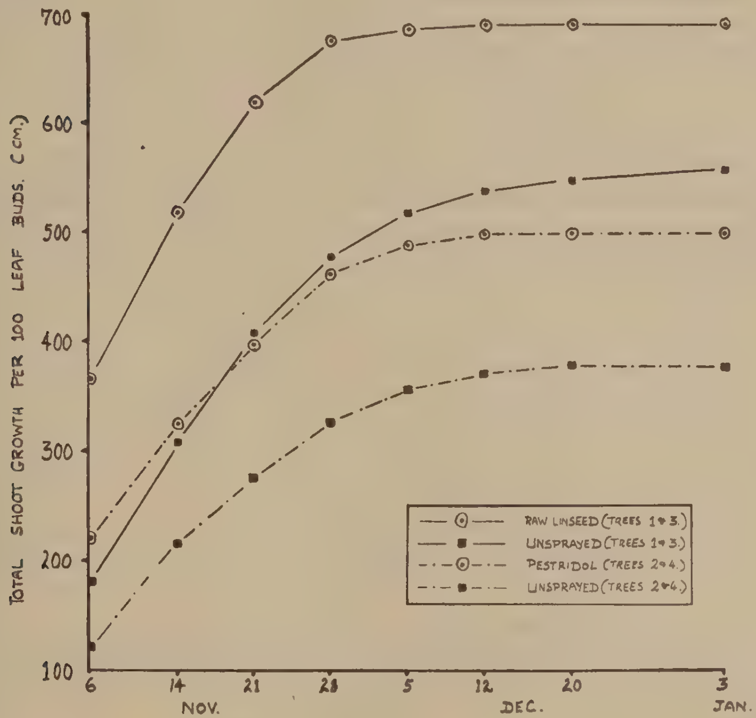

FIG. 2.

The effect of Raw Linseed Oil and Pestridol Dormant Sprays on shoot growth of the Bon Chrêtien Pear.

(c) *On leaf area.* Spur leaves were sampled on three dates from the two Bon Chrêtien trees half sprayed with raw linseed oil, and on one date from the two trees treated with Pestridol. The leaves were taken from the five spurs on each tree-half from which fruit buds were collected for dissection. The area of the leaves was measured in square inches with a planimeter, the results obtained being assembled in Table II. These show that spurs on two-year-old branches on the sprayed halves developed more leaves and a greater total leaf area per spur than those on the unsprayed tree-halves. When it is taken into account that these winter oil sprays also lead to increased spur bud formation

per unit length of young and old wood, it becomes evident that such treatments increase the total leaf surface of sprayed trees. In view of the findings of workers such as Vyvyan and Evans (14), Magness, Overley and Luce (7), Harley, Masure and Magness (4), Aldrich and Work (1), Swarbrick (12) and Thies (13), this increase in leaf area on sprayed trees seems to be an important factor in promoting fruit bud formation.

Taking the results of the Stellenbosch experiment as a whole, the conclusion is reached that both the winter oils tested had a beneficial effect on growth, leaf development and fruit bud formation in the Bon Chrêtien pear.

(2) GROOT DRAKENSTEIN RESULTS.

This experiment was conducted at Groot Drakenstein where a uniform block of thirty-five-year-old Bon Chrêtien pears was selected. The trees were on *communis* seedling stocks, and all had received similar cultural treatment prior to this experiment. The arrangement of the trees is shown in Fig. 3.

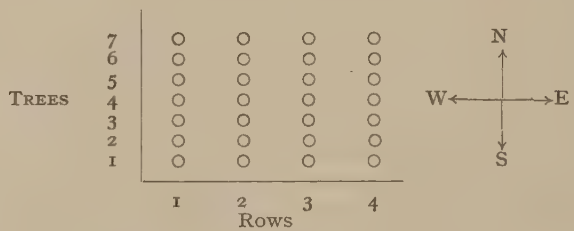

Fig. 3.
Plan of Bon Chrêtien Block at Groot Drakenstein.

On August 21st, 1935, the trees were sprayed with a power sprayer, the following winter oil emulsions being applied:—

Rows.	*Trees.*	*Treatment.*
1	1-5	Raw linseed oil emulsion 5% (casein-ammonia stock emulsion, approx. 80% oil by weight).*
2	1-5	Seal oil emulsion 5% (approx. 80% oil by weight)*.
3	1-5	Kleenup 5% (proprietary mineral oil emulsion, approx. 80% oil by weight).
4	1-5	Kleenup 3%.
1-4	6	Unsprayed.
1-4	7	3% Kleenup plus 3% Lime Sulphur.

These winter oils had all led to a marked increase in the 1936 crop, as is shown in Table III.

* Kindly supplied by "African Explosives & Industries Ltd.," Somerset West.

TABLE III.

Crop records of Bon Chrêtien Pear Trees, 1936.

Treatment.	Production.	Percentage Increase over Unsprayed Trees.
Raw Linseed Oil 5%	9·6 boxes per tree	92%
Seal Oil 5%	9·2 ,, ,, ,,	84%
Kleenup 5%	11·2 ,, ,, ,,	124%
Kleenup 3%	6·8 ,, ,, ,,	36%
Kleenup 3% plus Lime Sulphur 3%	8·4 ,, ,, ,,	68%
Unsprayed	5·0 ,, ,, ,,	

(Boxes averaged 32 lbs. nett weight of fruit.)

The oil-sprayed trees blossomed earlier and more regularly than and started growth before the unsprayed trees. It was thought that the more prolific blossoming of the treated trees directly affected their cropping. Bud mite (*Eriophyes pyri*) may have caused more damage to the later-blossoming fruit buds on the unsprayed trees, but this pest appeared to do little damage in this orchard during the 1935-36 season, owing to effective control in previous years.

During the winter of 1936, when defoliation was complete, records of fruit bud formation were taken on these trees. A number of main branches were selected at random on the northern and southern sides of each tree and the total growth of young and old wood on these branches was measured in centimetres. The number of fruit buds formed was also recorded. A summary of the results obtained is given in Table IV. They show that the various oil sprays stimulated the formation of fruit buds both on shoots and older wood.

TABLE IV.

The effect of various winter oil sprays on fruit bud formation in Bon Chrêtien Pears.

Treatment.	No. of Trees.	Total Shoot Growth in cm.	No. of Axial Fruit Buds on Shoot Growth.	% of Axial Fruit Buds formed per 100 cm. of Shoot Growth.	Total length of Old Wood in cm.	No. of Spur Buds on Old Wood.	Mean percentage of Spur Buds formed per 100 cm. of Old Wood and Standard Errors.
Raw Linseed Oil 5%	5	7483	629	8·40	7877	560	7·10 ± ·389
Seal Oil 5% ..	5	6491	576	8·87	7735	614	7·93 ± ·574
Kleenup 5% ..	5	7385	540	7·31	7938	456	5·74 ± ·424
Kleenup 3% ..	5	6528	477	7·30	7288	354	4·85 ± ·539
Kleenup 3% + Lime Sulphur 3%	4	5322	376	7·06	6073	229	3·77 ± ·545
Unsprayed	4	6033	340	5·63	6255	133	2·12 ± ·215

A further indication is that, with the exception of the 3% Kleenup plus 3% lime sulphur spray, all treatments led to a significant increase in spur fruit bud formation. The latter point is important, as with the Bon Chrêtien pear it is preferable that the greater portion of the crop should be borne on spurs.

The results confirm the findings of Black (2) who found that in the Bon Chrêtien pear winter oil sprays increased the bearing capacity of the tree owing to the formation of more fruit buds on both spurs and shoots.

SUMMARY.

The dormant application of 5% emulsions of raw linseed oil, and a proprietary oil, Pestridol, at Stellenbosch in 1935, advanced the initiation of fruit bud formation on these trees by 2-3 weeks.

Tree-halves treated with these two sprays gave a better performance than unsprayed halves in that:—

(a) less leaf buds remained dormant,

(b) more fruit spurs were formed,

(c) shoot growth was increased,

(d) greater leaf area and more leaves developed per fruit spur on two-year-old wood.

At Groot Drakenstein the application of dormant sprays of raw linseed oil (5%), seal oil (5%) and Kleenup (5% and 3%) in 1935, led to an increase both in crop and in fruit bud formation on young and old wood.

ACKNOWLEDGMENTS.

The author wishes to thank Mr. M. W. Black for his collaboration in the work done on the effect of winter oil sprays at Stellenbosch ; Messrs. H. E. V. Pickstone & Son, of Groot Drakenstein, for placing trees at his disposal and Mr. T. E. Micklem who assisted in the spraying and the recording of crops at Groot Drakenstein. Also the Imperial Bureau of Horticulture and Plantation Crops for kindly undertaking the responsibility for seeing the papers through the press.

REFERENCES.

(1) *Aldrich, W. W.* and *Work, R. A.* Effect of leaf-fruit ratio and available soil moisture in heavy clay soil upon amount of bloom in pear trees. Proc. Amer. Soc. Hort. Sci., 1934, **31**, 57.

(2) *Black, M. W.* Deciduous fruit investigations. II. Some physiological effects of oil sprays upon deciduous fruit trees. U. of S.A. Dept. Agric. 1936, Sci. Bull. 154.

(3) *Haller, M. H.* and *Magness, J. R.* Relation of leaf area and position to quality of fruit and to fruit bud differentiation in apples. U.S. Dept. Agric., 1933, Tech. Bull. 338.

(4) *Harley, C. P., Masure, M. P.* and *Magness, J. R.* Fruit thinning and biennial bearing in yellow Newtown apples. Proc. Amer. Soc. Hort. Sci., 1933, **30**, 330.

(5) *Hooker, H. D.* and *Bradford, F. C.* Localization of the factors determining fruit bud formation. Mo. Agric. Expt. Sta., 1921, Res. Bull. 47.

(6) *Levering, S. R.* Leaf weight per spur correlated with the yield of the Baldwin apple. Proc. Amer. Soc. Hort. Sci., 1933, **30**, 303.

(7) *Magness, J. R., Overley, F. L.* and *Luce, W. A.* Relation of foliage to fruit size and quality in apples and pears. State Coll. of Wash. Agric. Expt. Sta., 1931, Bull. 249.

(8) *Mally, C. W.* Raw linseed and seal oil for controlling irregular blossoming and foliation in fruit trees. U. of S.A. Dept. of Agric., 1934, Bull. 125.

(9) *Maney, T. J.* and *Plagge, H. H.* Factors which influence the production and growth of fruit buds on the apple. Proc. Amer. Soc. Hort. Sci., 1921, **18**, 100.

(10) *Roberts, H. H.* Effect of defoliation upon blossom bud formation. Univ. Wis. Agric. Expt. Sta., 1923, Res. Bull. 56.

(11) *Summers, F.* The factors governing bud formation: A chapter of plant physiology. New Phytol., 1924, **23**, 20, 78, 113.

(12) *Swarbrick, T.* Some observations upon the leaf area on spurs on biennially bearing apple trees. Ann. Rpt. Agric. & Hort. Res. Sta., Long Ashton, 1928, 23.

(13) *Thies, W. H.* Effect of defloration on spur leaf area in the McIntosh apple. Proc. Amer. Soc. Hort. Sci., 1933, **30**, 309.

(14) *Vyvyan, M. C.* and *Evans, H.* The leaf-relations of fruit trees. Journ. Pom. & Hort. Sci., 1932, **10**, 228.

(15) *Wiggans, C. B.* The influence of certain environmental and cultural conditions on fruit bud formation of pear and apricot. Journ. Agric. Res., 1925, **31**, 865.

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SOME RESULTS OF EXPERIMENTS IN BREEDING BLACK CURRANTS

PART II. FIRST CROSSES BETWEEN THE MAIN VARIETIES

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It is now just eight years since the first part of this paper appeared (5) giving details of studies, extending over about ten years, of families obtained from the self pollination of some of the principal black currant varieties. While no attempt was made at that time to give a definite genetic interpretation of the inheritance of characters, the types of seedling encountered in such families and the proportions in which the various characters segregated were broadly indicated, in the hope that such information might prove of value to the breeder of new varieties.

At the conclusion of the work on the self pollinated families, it was decided to proceed to a study of families from crosses between the same varieties. French Black, Seabrook's Black, Goliath, Boskoop Giant and Baldwin were therefore intercrossed in every possible combination and their progeny has been subjected to careful observation over a period of seven years. In the light of the confirmatory information provided by these seedlings, it has been possible to attempt a genetical analysis for a few characters. Since, however, many of the character differences are extremely slight and there is unquestionably some variation from year to year, a detailed analysis has not been attempted for most of them. Such information on a simple diploid plant like the black currant can have little interest for the geneticist and cannot further the work of the practical breeder, since most of the characters that have been studied in detail are of slight economic importance. Many of the character differences that have been dealt with are those upon which Hatton (3) based his classification of black currant varieties. While many of them are not of importance to the raiser of new varieties, they are usually those in which the principal varieties differ most markedly.

Details of the methods employed in this work have already been given (5).

I. SEEDLINGS OF FRENCH BLACK × SEABROOK'S BLACK.

French Black × Seabrook's Black. 58 seedlings.
Seabrook's Black × French Black. 6 seedlings.

When Seabrook's Black was used as the female parent and crossed with pollen of French Black, some evidence of incompatibility was obtained. Of

41 flowers pollinated, only 9 (22 per cent.) set fruits, whereas when French Black was used as female, of 46 flowers pollinated, 25 (54 per cent.) set fruits. There was also a marked difference in the germination of the seeds. Of the 53 seeds from the cross in which Seabrook's Black was the female parent, only 6 (11 per cent.) germinated, while of the 340 seeds from the cross in which French Black was the female, 170 (50 per cent.) germinated.

1. LEAF CHARACTERS.

Descriptions have already been given of the abnormal types of leaf which occur among seedlings from self-pollinated families of varieties in the French group. It was shown that while Seabrook's Black, when selfed, gave rise to plants with leaves of four distinct types, designated SR (normal), Sr, sR and sr, in the ratio of 9:3:3:1, only two types occurred among seedlings of selfed French Black. They resembled very closely the SR and Sr types found among the Seabrook's Black seedlings and were in the ratio of approximately 3 SR to 1 Sr. The factorial constitution of Seabrook's Black appears to be SsRr while that of French Black is probably SSRr. A cross between the two varieties would therefore be expected to give seedlings in the proportions of 3 SR (normal) to 1 Sr. Actually all six of the seedlings in the family in which Seabrook's Black was the female were of the normal leaf type, but among the fifty-eight individuals from the cross in which French Black was the female eight were of the Sr leaf type. This is less than the number to be expected on theoretical grounds, but the discrepancy is not serious.

The seedlings of the SR (normal) leaf type had leaves of medium size, deep green colour, with a long terminal lobe and fairly deep lobe sinus. The basal lobe was invariably shallow or flat (see Fig. 1). They were very similar in all general respects to those of the parent varieties. The leaf stalks were either deep red, tinged with pink, or green ; and the three classes occurred among all the seedlings in proportions which approximated fairly closely to a 1:2:1 ratio. Distinct differences were observed in the angle at which the leaf blades were held in relation to the leaf stalks (see Fig. 2). In most of the seedlings they were downturned, but there were many in which they were curved over and, in three seedlings, they were held out horizontally. These classes did not occur in any simple Mendelian proportions.

2. VIGOUR.

All the seedlings were moderately vigorous.

3. BUD COLOUR.

Both French Black and Seabrook's Black have deep red buds but they are probably not genetically identical since, when selfed, Seabrook's Black gave some seedlings with green buds, while French Black did not. Among these

seedling crosses, approximately one-quarter had green buds while those of the remainder were either deep red like their parents, or of an intermediate type, with buds less intensely pigmented. The colour of the buds has been found to fluctuate considerably from one season to another, and it was difficult to obtain data of sufficient accuracy for genetical analysis. It seems probable that some mechanism for the inhibition of colour is involved, which may become partially inoperative under certain seasonal conditions.

4. Form of the Buds.

In both French Black and Seabrook's Black the buds are conical in shape, somewhat protruding outwards from the shoots and with tightly wrapped scales. Among the seedlings, conical, intermediate and round buds occurred in a ratio of 9:6:1, suggesting that bud shape may be controlled by a pair of factors cumulative in their effect. Seedlings with protruding buds comprised rather more than three-quarters of the whole, and those with compactly wrapped bud scales occurred in about the same proportions.

5. Season of Coming into Leaf.

All these seedlings were among the latest to come into leaf.

6. Flower Characters.

The flowers borne by these seedlings were usually of medium size and of a somewhat pale colour, although a number occurred with perianths tinged bright red. It will be recalled that "running off", or the failure of some varieties to set fruits, was found by Hatton and his colleagues (6) to be associated with the position of the style in the flower in relation to that of the anthers. Where the styles were much above the level of the anthers, the setting of the fruit was unsatisfactory. Observations were made upon the relative position of the styles and anthers of the flowers of these seedlings. There was considerable variability and only an estimate could be attempted. It was found that 70 per cent. bore flowers with styles above the level of the anthers while the remaining bushes had flowers with styles and anthers at the same level.

Only about 14 per cent. had long flowering racemes, about 50 per cent. had racemes of intermediate length, while those of the remainder were short. The racemes were held somewhat stiffly outwards in a horizontal direction in the majority, but in about 30 per cent. the racemes were waved. Thirteen per cent. of the bushes blossomed early, 55 per cent. were midseason and 32 per cent. late. Nearly 60 per cent. had an average of seven flowers to the raceme, 30 per cent. had an average of nine, while a few individuals occurred with an average of eleven, and a single bush had as many as thirteen flowers to the raceme.

7. FRUIT CHARACTERS.

Of these seedlings, only eight bore large fruits, those of the remainder being either of medium size or small. About a quarter of the bushes bore early ripening fruits, those of the rest being midseason. Only two bushes were found on which the fruits were late ripening. The set of fruit was never particularly good, partly due to spring frosts and partly to "running off", and was only fair or poor in all of them. In flavour the fruits were either intermediate or definitely acid and only three seedlings bore fruits of a sweet flavour. The fruits on about three-quarters of the bushes had tough skins, those of the remainder being tender.

8. CROPPING.

The crops obtained from these seedlings have been only small. During their sixth season they averaged 0·7 lb. per bush and in their seventh season, 1·1 lb. A few seedlings were found with crops markedly above this average, but none of sufficient promise to merit further trial was discovered.

II. SEEDLINGS OF FRENCH BLACK × BOSKOOP GIANT.

French Black × Boskoop Giant. 46 seedlings.
Boskoop Giant × French Black. 87 seedlings.

There was no evidence of any incompatibility when these two varieties were intercrossed, but a marked difference was noted in the germination of the seeds. Of 914 seeds, from the cross in which French Black was the female, 466 (51 per cent.) germinated, while of 1,596 seeds from that in which Boskoop Giant was the female only 104 (6·5 per cent.) germinated.

1. LEAF CHARACTERS.

The leaves of these seedlings were usually of medium size to large and deep green. The lobe sinus was of medium depth and the serrations small and regular. The basal sinus was either of medium depth or shallow, only a few individuals being noted with a somewhat deep leaf sinus. About half the seedlings had downturned leaves, while those of the rest were curved over. The petioles were either deep red, tinged with pink, or green; and the seedlings with tinged petioles were about twice as numerous as those with deep red or green petioles.

2. VIGOUR.

All the seedlings were vigorous and many were spreading in habit, like Boskoop Giant.

3. Bud Colour.

Seedlings with pinkish buds predominated in these families, although about 35 per cent. had deep red buds. Only a few seedlings with green buds were found.

4. Form of the Buds.

Slightly less than a quarter of these seedlings had conical buds, while about the same proportion had round buds. The buds of the remaining bushes were intermediate. The bud scales on all the seedlings were tightly wrapped, with the exception of six which had somewhat ragged scales. Protruding buds occurred on about three-quarters of the bushes.

5. Season of Coming into Leaf.

While French Black is very late in coming into leaf, Boskoop Giant is fairly early. No seedling early in season of bud-break was discovered, there being almost exactly equal proportions of midseason and late seedlings.

6. Flower Characters.

The flowers borne by these seedlings were usually of medium size and were frequently tinged bright red. About one-half of the bushes were early in season of blossoming, most of the remainder being midseason. Only two seedlings were recorded as late in time of flowering. Approximately 73 per cent. of the bushes had flowers with styles above the level of the anthers, bushes with flowers with level styles comprising only 27 per cent. of the whole. About one-half had an average of nine flowers per raceme, about 30 per cent. an average of seven, and there was an average of eleven per raceme on the remaining seedlings. Long flowering racemes occurred on less than a quarter of the seedlings. Rather more than one-half had racemes of medium length and those of the rest were short. The racemes of about half were held stiffly outwards in a horizontal direction and in the other half they were somewhat waved.

7. Fruit Characters.

Most of the seedlings bore fruits of medium size. About 10 per cent. of them had large fruits in their sixth year, while about 7 per cent. had small fruits. They were predominantly early in season of ripening their fruits and no seedlings with late fruits were recorded. By far the larger proportion were described as badly "run off" in their sixth and seventh years. A few set a fair proportion of their flowers but none was found in which the set was good. Most of the seedlings were neither markedly sweet nor acid in flavour, but about 9 per cent. had sweet flavoured fruits and those of about 33 per cent. were decidedly acid. Approximately one-half of the bushes had fruits with tough skins while, in the other half, the skins of the fruits were tender.

8. Cropping.

In their sixth season these seedlings produced, on the average, slightly less than half a pound of fruit and in their seventh season, 0·65 lb. per bush. Four seedlings bore more than three times the average amount of fruit in their sixth year, but none of them showed any outstanding combination of desirable characters. Large berries and long fruiting racemes were found together in five seedlings, four of which had fruits early in season of ripening. The crops recorded on these bushes were not outstandingly large, but three of them had more than twice the average for the whole family at six years old. Two have been thought worthy of retention for further trial.

III. SEEDLINGS OF FRENCH BLACK × GOLIATH.

French Black × Goliath. 53 seedlings.
Goliath × French Black. 53 seedlings.

Although these two varieties were completely interfertile when crossed, the germination of the seed was poor in both families. When French Black was used as the female parent, 112 seedlings were obtained from 893 seeds, a germination of only 12 per cent. When Goliath was used as the female, 885 seeds gave 213 plants, a germination of 24 per cent.

1. Leaf Characters.

The leaves of the seedlings in these families were usually of medium size with long terminal lobes and regular serrations. In colour they were variable. In most cases the leaves were dark green but in a few seedlings the foliage was pale, yellow green. The leaf sinus was either intermediate or shallow, the former class being only about half as numerous as the latter. No seedlings with a deep leaf sinus were found. About half the bushes had green leaf stalks, a rather smaller number had them tinged with pink, while only seven had deep red leaf stalks. About half the seedlings had downturned blades, the rest being curved over.

2. Vigour.

In general, these seedlings were fairly vigorous and frequently made compact bushes with stout, erect shoots.

3. Bud Colour.

Seedlings with deep red buds comprised about one-third of the total in these families, about one-half had buds of an intermediate, pinkish colour, while the rest had green buds.

4. Form of the Buds.

Most of the seedlings had conical buds, although there was a large class with buds of an intermediate shape and a few bushes were found with round buds. The actual proportions of the three types found, 69:39:8, approximated very closely to a 9:6:1 ratio. About three-quarters had compactly wrapped bud scales, those of the rest being ragged. Protruding buds also occurred on about three-quarters of the bushes, the buds on the rest being closely adpressed to the shoots.

5. Season of Coming into Leaf.

These seedlings were either midseason or late in coming into leaf and the two classes occurred in about equal proportions. Early leafing bushes were not found.

6. Flower Characters.

The flowers were usually somewhat small. In most cases they were of a pale colour only faintly tinged with pink, but a few individuals occurred in which the flowers were deeply tinged bright red. Observations on the relative position of the styles showed that approximately 70 per cent. had them protruding well above the anthers, while those of the remaining 30 per cent. were on a level. Only one seedling was found with long flowering racemes, those of the majority being of intermediate length. On more than 40 per cent. of the bushes the racemes were short. There was an average of nine flowers per raceme on about 60 per cent. of the bushes and an average of seven on nearly 40 per cent. A single bush was found with an average of eleven flowers per raceme and another with as few as six. The racemes of about half the bushes were held out horizontally while those of the rest were waved.

7. Fruit Characters.

In season of ripening their fruit, most of these seedlings were midseason although a few were early and a few late. The fruits were usually of medium size, although those of about 20 per cent. were large and about the same proportion small. None of them set a large percentage of the flowers in the seasons during which they were under observation. About 70 per cent. of them had fair sets while on the rest the sets were poor. The sweet flavour of the variety Goliath had been inherited by only about 15 per cent. and the acid flavour of French Black by about 27 per cent. The flavour of the fruits of the rest was neither outstandingly sweet nor acid. Rather more than 70 per cent. of the bushes had fruits with tough skins while on the remaining bushes the fruits had tender skins.

8. CROPPING.

At six years old, the seedlings in these families averaged about 1 lb. of fruit per bush and at seven years old, 1·31 lb. Only two bushes occurred with crops notably above the average, but as they showed no other desirable characters they were not considered worthy of further trial.

IV. SEEDLINGS OF FRENCH BLACK × BALDWIN.

French Black × Baldwin. 56 seedlings.
Baldwin × French Black. 56 seedlings.

No evidence of incompatibility was discovered in these crosses; the fruits contained numerous seeds which germinated well.

1. LEAF CHARACTERS.

The leaves of these seedlings were of medium size or large and mid or deep green in colour, with a long terminal lobe and small, regular serrations. About one-half had leaf sinuses of intermediate depth while those of the rest were shallow. Only four bushes had deep sinuses. About one-quarter had deep red petioles, in the remainder they were pinkish or green in about equal proportions. Slightly more than three-quarters of the seedlings had downturned blades while those of the rest were curved over.

2. VIGOUR.

All these seedlings were vigorous in growth.

3. BUD COLOUR.

About one-half of these seedlings had buds which were pinkish or intermediate in colour, about 16 per cent. had deep red buds, while the remaining 34 per cent. had green buds.

4. FORM OF THE BUDS.

The buds were conical in most of the seedlings but a fairly large class occurred in which they were intermediate. Only nine bushes were recorded as having rounded buds. Seedlings with compact and with ragged bud scales occurred in about equal proportions, while all the seedlings except eight had buds protruding from the shoot.

5. SEASON OF COMING INTO LEAF.

About a quarter of these seedlings were early in season of bursting into leaf, one-half were midseason and one-quarter late.

6. Flower Characters.

The flowers produced by these seedlings were usually somewhat small and pale. Rather more than one-half bore flowers the styles of which were above the level of the anthers, in the rest the styles were on a level with them. Long flowering racemes were borne by just a quarter of the bushes, in one-half they were of intermediate length while those of the remaining bushes were short. There was an average of nine blossoms to the raceme on about one-half of the seedlings and rather less than one-half had an average of seven, whilst a few bushes had an average of eleven to the raceme. With the exception of six bushes, which had trusses held stiffly outwards, all these seedlings had waved racemes.

7. Fruit Characters.

Most of these seedlings had fruits of medium size and midseason in time of ripening. A few, slightly more than one-eighth, had large, and about the same proportion, small fruits. About one-quarter were early in season of ripening and a very few bushes, about 6 per cent., were late. The setting of the fruit was never particularly good on these bushes and with two exceptions was only fair to definitely poor. Seedlings with fruits of intermediate flavour and tough skins preponderated.

8. Cropping.

These seedlings averaged about 0·9 lb. of fruit per bush in their sixth season and 1·33 lb. in their seventh. Several bore crops considerably above this average and a few have been deemed worthy of further trial. It is to be noted that although seedlings with long racemes, large fruits and sweet flavours were found, no seedling occurred combining these characters.

V. SEEDLINGS OF SEABROOK'S BLACK × BOSKOOP GIANT.

Seabrook's Black × Boskoop Giant. 53 seedlings.
Boskoop Giant × Seabrook's Black. 56 seedlings.

These two varieties were perfectly interfertile when crossed and considerable seed was obtained, but the seed germinated very badly. In the cross in which Seabrook's Black was used as the female, of 754 seeds sown, only 95 (13 per cent.) germinated and when Boskoop Giant was used as the female 941 seeds gave 56 seedlings, a germination percentage of 6.

1. Leaf Characters.

In general, the leaves were large, dark green, with long terminal lobes and small, regular serrations. The lobe sinus was usually of medium depth although

occasional seedlings occurred with leaves with a deep lobe sinus. An approximately equal number of bushes had leaves with a shallow leaf sinus and one of medium depth, but no seedlings were found with deep leaf sinuses. Exactly one-half of the seedlings had leaf stalks tinged pink while those of about one-quarter were deep red and of the remaining quarter green. There were about three times as many seedlings with downturned blades as with them curved over the petioles.

2. Vigour.

All these seedlings were vigorous and many had a somewhat spreading habit of growth.

3. Bud Colour.

The buds of rather less than half these seedlings were deep red, those of an approximately equal number were of an intermediate, pinkish colour, while there were eight bushes with green buds.

4. Form of the Buds.

Twenty-six seedlings had conical buds, those of sixty-four were intermediate while nineteen bushes had round buds. Seedlings with compact and with ragged bud scales occurred in a ratio of about seven of the former to one of the latter while there were just about four seedlings with buds protruding from the shoots to every seedling with buds closely adpressed to them.

5. Season of Coming into Leaf.

Seedlings early in season of coming into leaf were not found in these families. About one-half were midseason and the rest late.

6. Flower Characters.

The flowers were of medium size to large and frequently bright red. On about 70 per cent. the styles of the flowers were above the level of the anthers, those of the rest being on a level. About one-third were early in season of blossoming, rather more than one-half were midseason and the rest were late. Long flowering racemes were recorded on about one-third of the bushes, on rather more than one-half they were of medium length, while the remainder had short racemes. On about half the bushes, the racemes were held stiffly outwards, while, on the other half, they were waved. There was an average of about seven flowers per raceme on 41 per cent., of nine on 42 per cent. and of eleven on the rest.

7. Fruit Characters.

Less than a quarter of these seedlings bore large fruits while those of three-quarters were of medium size. Small fruits were recorded on very few bushes.

The fruits were early in season of ripening on about three-quarters of the bushes, while those of the rest were midseason. Bushes with fruits late in season were not found in these families. Sets were usually described as only fair or poor, but a few seedlings were found upon which the sets were fairly good. Sweet flavoured fruits occurred only rarely, most of the bushes having fruits either sub-acid or definitely acid. About equal numbers had fruits with tough and with tender skins.

8. CROPPING.

At six years old, these seedlings yielded a crop of about half a pound of fruit per bush and, at seven years old, the crop amounted to 0·57 lb. Several bushes were found with considerably more than the average quantity of fruit and two had more than five times the average in their sixth year. Several have been thought promising enough to retain for further trial.

VI. SEEDLINGS OF SEABROOK'S BLACK × GOLIATH.

Seabrook's Black × Goliath. 49 seedlings.
Goliath × Seabrook's Black. 55 seedlings.

There was no evidence of any incompatibility between these two varieties when they were intercrossed, although there was some difference in the germination of the seed. Of 266 seeds sown, in the cross of which Seabrook's Black was the female parent, 123 (46 per cent.) germinated, whereas, when Goliath was used as the female, of 1,229 seeds sown, 302 (24 per cent.) germinated.

1. LEAF CHARACTERS.

In colour, the leaves of these seedlings were usually medium or deep green, but a few occurred with rather pale green foliage. The leaves were of medium size or, more rarely, large ; the terminal lobes were long and the serrations regular. The lobe sinus was usually of medium depth. The basal sinuses were either intermediate in depth or shallow ; deep basal sinuses were not found. About half had leaf petioles tinged pink, those of about one-tenth deep red, and the rest green. About half the bushes had downturned blades, those of the other half being curved over the petioles.

2. VIGOUR.

All the seedlings were fairly vigorous and many had the compact, erect habit of Goliath.

3. BUD COLOUR.

About 40 per cent. of the bushes had red buds and those of about an equal number were intermediate, or pinkish, in colour. The remaining 20 per cent. had green buds.

4. Form of the Buds.

The buds were either conical or intermediate in shape, the former being slightly more numerous. Only about one-sixteenth had round buds. Slightly less than three-quarters had tightly arranged bud scales, those of the rest being ragged. Bushes with protruding buds were more numerous than those with buds adpressed to the shoots by about three to one.

5. Season of Coming into Leaf.

None of these seedlings was early in season of coming into leaf. Half were midseason and the rest late.

6. Flower Characters.

The flowers were usually somewhat small and pale in colour although a few seedlings occurred with bright red flowers. Slightly more than one-half bore flowers the styles of which were above the level of the anthers, those of the rest being on a level. In season of blossoming, about one-half were early, one-quarter midseason and one-quarter late. Seedlings with long flowering trusses were not found. One-half had trusses of medium length and one-half had short trusses. In three-quarters, the racemes were waved while, in the rest, the racemes were held stiffly outwards. About half had an average of seven flowers per truss and half an average of nine flowers per truss. On a few bushes there were only six flowers to the truss.

7. Fruit Characters.

Considerably less than a quarter of these seedlings bore large fruits, those of most being of medium size. Upon a few bushes the fruits were definitely small. On rather more than half, the set of fruit was fairly good in their sixth season while that of the rest was poor. Really good sets were found on only about 2 per cent. of the bushes. The flavour of the fruit was sweet on only about 4 per cent., quite acid on 36 per cent., while that of the remainder was intermediate. About three-quarters of the seedlings had fruits with tough skins, those of the rest being tender.

8. Cropping.

At six years from the seed, these seedlings bore an average of 0·7 lb. of fruit per bush and at seven years the crop was 1·21 lb. per bush. Several gave crops considerably in excess of these averages. No seedlings of sufficient merit to warrant their retention for further trial were found.

VII. SEEDLINGS OF SEABROOK'S BLACK × BALDWIN.

Seabrook's Black × Baldwin. 52 seedlings.
Baldwin × Seabrook's Black. 56 seedlings.

These varieties were completely interfertile. The fruits of Baldwin, when pollinated with Seabrook's Black, gave an average of seventy seeds per fruit, but the fruits of Seabrook's Black, when pollinated with Baldwin, contained fewer seeds. Only 20 per cent. of the seeds germinated, when Baldwin was used as the female plant, whereas 55 per cent. germinated when Seabrook's Black was so used.

1. Leaf Characters.

These seedlings usually had large, deep green leaves with long, broad terminal lobes, often somewhat coarse serrations with a deep, or moderately deep, lobe sinus. The basal leaf sinus was shallow in most of them, although bushes with a sinus of intermediate depth occurred frequently. Slightly more than three-quarters had leaf petioles tinged with pink, while the rest had deep red petioles, with the exception of four bushes, on which the petioles were green. About three-quarters had downturned blades, those of the rest being curved over the petioles.

2. Vigour.

All these seedlings were moderately vigorous.

3. Bud Colour.

Deep red buds were found on only about 10 per cent. of these seedlings; those of about one-half were tinged with pink and those of the rest were green.

4. Form of the Buds.

Most of these bushes had conical buds, but those of rather more than one-third were intermediate. Only three seedlings were found with round buds. Just about one-half had tightly wrapped bud scales, those of the rest being ragged and loosely arranged. All the bushes had buds protruding from the shoots with the exception of three in which the buds were closely adpressed.

5. Season of Coming into Leaf.

About a quarter of these bushes were early in season of coming into leaf, one-half were midseason and the rest late.

6. Flower Characters.

The flowers were usually of medium size and were frequently tinged bright red. About three-quarters bore flowers with styles protruding above the

anthers, while those of the rest were level. In season of blossoming, most of the bushes (actually only slightly less than three-quarters) were early, while the rest were midseason. Seedlings with long flowering trusses did not occur with great frequency, only about 14 per cent. being so described. Almost three-quarters had racemes of medium length while those of the rest were short. With the exception of five bushes, in which the racemes were held stiffly outwards, the racemes on all the bushes were waved. There was an average of seven flowers to the raceme on about one-third ; more than one-half had an average of nine, while the rest had an average of eleven.

7. Fruit Characters.

The fruits were usually of medium size, but in about a quarter of the bushes they were large. The fruits of about three-quarters were midseason in time of ripening and those of the rest were early. Only a very few bushes with late ripening fruits were found. The fruits were either sub-acid or acid in flavour, sweet flavoured fruits occurring on only about 11 per cent. Just three-quarters had fruits with tough skins, those of the rest being tender. The sets were only fair or definitely poor throughout and only six bushes were found upon which the sets were good.

8. Cropping.

In their sixth season these seedlings bore an average of exactly three-quarters of a pound of fruit per bush and in their seventh season the crop was 1·8 lb. per bush. Several seedlings gave crops considerably in excess of these averages and a number have been considered worthy of further trial.

VIII. SEEDLINGS OF BOSKOOP GIANT × GOLIATH.

Boskoop Giant × Goliath. 55 seedlings.
Goliath × Boskoop Giant. 53 seedlings.

These two varieties proved completely interfertile and a large proportion of the pollinated flowers set fruits which contained abundant seeds. There was, however, considerable difference in the germination of the seeds in the two crosses. Where Boskoop Giant was used as the seed parent, only 5 per cent. of the seeds germinated, but when Goliath was used, 33 per cent. of the seeds did so.

1. Leaf Characters.

The leaves were usually deep green, although a small proportion of seedlings occurred in which the leaves were pale green. They were of medium size or large, with long terminal lobes and small, regular serrations. About a

quarter had leaves with a deep basal leaf sinus and, in about the same proportion, the leaves had shallow basal sinuses. In the remainder, the sinuses were of intermediate depth. The leaf petioles were deep red in about 27 per cent., those of 50 per cent. were tinged with pink, the remaining bushes having green petioles. Downturned blades were found on 38 per cent., the blades of the rest being curved over the petioles.

2. Vigour.

All the seedlings were moderately vigorous and a number had the compact, erect habit of Goliath.

3. Bud Colour.

Deep red buds occurred on about 20 per cent. of these bushes, those of 58 per cent. were tinged with pink while the rest had green buds.

4. Form of the Buds.

The buds of 27 per cent. were conical, buds of intermediate shape occurred on 55 per cent. and those of the remainder were rounded. About three-quarters had buds with tightly wrapped scales while those of the rest were loose. The buds were protruding from the shoots in 82 per cent., those of the rest being closely adpressed.

5. Season of Coming into Leaf.

There were eleven seedlings early in season of coming into leaf, seventy-four were midseason and twenty-three late.

6. Flower Characters.

The flowers were usually rather small and pale. The flowering racemes were either of medium length or short in about equal proportions. Only eleven seedlings were found with long racemes. Most of the bushes were early in season of blossoming but about one-third were midseason and one-seventh were late. About one-half had styles on a level with the anthers, those of the rest being well above them. The racemes were waved on rather less than three-quarters and held out horizontally on the rest. There was an average of seven flowers to the raceme on about half the seedlings. Almost as many had an average of nine, while a few bushes occurred with as few as six and as many as eleven.

7. Fruit Characters.

Most of the seedlings had fruits of medium size, although on about 30 per cent. they were large. Small fruits were found on only three bushes. The fruits were mostly early in season of ripening but on about 35 per cent. they

were midseason and there were three with late fruits. The sets were throughout only fair or poor. The fruits were usually sub-acid but, on 10 per cent., they were definitely sweet and on about 30 per cent. quite acid. Tough skins were found on the fruits of about half the bushes, those of the rest being tender.

8. Cropping.

In their sixth season from the seed, these seedlings averaged 0·62 lb. of fruit per bush and in their seventh season the average was 1·42 lb. Several bushes were found with crops considerably above these averages, but none was sufficiently good to merit selection for further trial.

IX. SEEDLINGS OF BOSKOOP GIANT × BALDWIN.

Boskoop Giant × Baldwin. 57 seedlings.
Baldwin × Boskoop Giant. 57 seedlings.

These two varieties were completely interfertile. Of the 1,142 seeds obtained from the cross in which Baldwin was used as the seed parent, only 60 (5 per cent.) germinated. Of the 1,893 seeds obtained when Boskoop Giant was the seed parent, 675 (35 per cent.) germinated.

1. Leaf Characters.

The leaves were generally large, deep green, with a long, broad terminal lobe and often somewhat coarse serrations. The leaf blades were frequently slightly upfolded along the edges and the lobe sinuses were of moderate depth. In about a quarter of the seedlings the basal leaf sinus was deep, 55 per cent. had a sinus of medium depth, while in the rest it was shallow. Most of the bushes had petioles tinged light pink but, in about 40 per cent., they were deep red. There were only ten seedlings in which they were completely green. About 65 per cent. had downturned blades, in the rest the leaves were curved over the petioles.

2. Vigour.

All the seedlings were fairly vigorous and many of them made large, spreading bushes.

3. Bud Colour.

Only about 8 per cent. of the bushes had deep red buds, in about one-half they were intermediate, or tinged with pink, while, in the rest, they were completely green.

4. Form of the Buds.

Conical buds occurred on about 36 per cent. of the seedlings, 54 per cent. had buds intermediate in shape while on the rest the buds were round. Approximately 70 per cent. had compactly wrapped bud scales, and in the rest the bud scales were loosely and raggedly arranged. All but ten had buds protruding from the shoot.

5. Season of Coming into Leaf.

About one-half of these seedlings were early in season of coming into leaf, about the same proportion were midseason, while there were only ten late seedlings.

6. Flower Characters.

The flowers were usually of medium size or, quite frequently, large, and were, in many instances, tinged a bright red. Rather more than three-quarters of the bushes bore flowers with styles protruding well above the level of the anthers and only in those of the remaining quarter were they found to be on a level. Most of the seedlings were early in time of blossoming, less than 10 per cent. were midseason and none were late. About half had long flowering racemes and an almost equal number had racemes of medium length. On only about 3 per cent. were definitely short racemes found. There was an average of nine flowers per raceme on about half the bushes, approximately 36 per cent. had an average of eleven, while there were seven on 7 per cent. and a single bush was found with as many as thirteen. Wavy racemes occurred on three-quarters of the bushes, while the racemes of the rest were held out stiffly in a horizontal direction.

7. Fruit Characters.

The fruits were large on only 30 per cent. of the seedlings, on 65 per cent. they were of medium size and on only 5 per cent. were they small. They were predominantly early in ripening. About 35 per cent. bore midseason fruits, but only two seedlings were found with late ripening fruits. The setting was never really satisfactory and, in general, it was only fair or definitely poor. Bushes with sweet flavoured fruits were of rare occurrence and in less than a quarter was the flavour acid. The fruits were mostly of an intermediate, sub-acid flavour. Those of about half the bushes had tough skins and in the rest the skins were tender.

8. Cropping.

The 114 bushes comprising these two families gave an average of slightly less than half a pound of fruit in their sixth season. In their seventh the

average was 2·1 lb. Two seedlings produced more than three times the average quantity of fruit in their sixth year, but neither of them was characterized by any particularly desirable combination of characters.

X. SEEDLINGS OF GOLIATH × BALDWIN.

Goliath × Baldwin. 56 seedlings.
Baldwin × Goliath. 56 seedlings.

Although these two varieties were completely interfertile, the seed obtained from the cross in which Goliath was the seed parent germinated rather better than that from the cross in which Baldwin was the seed parent. In the former, 1,361 seeds gave 456 seedlings, a germination of 33 per cent., whereas, in the latter, 2,132 seeds gave 241 seedlings, a germination of 11 per cent.

1. LEAF CHARACTERS.

Most of the seedlings in these two crosses had leaves of medium size, frequently pale green, with rather short lateral and long terminal lobes. The serrations of the leaf margins were usually small and regular. About 20 per cent. had leaves with a deep basal sinus, in 53 per cent. the sinus was of medium depth, while it was shallow in the rest. Deep red leaf stalks were found on 17 per cent. of the bushes, they were tinged with pink on 60 per cent., while in the rest they were completely green. Approximately one-half had down-turned leaf blades, those of the other half being curved over the leaf stalks.

2. VIGOUR.

All these seedlings were fairly vigorous and frequently of a compact habit of growth.

3. BUD COLOUR.

About three-quarters of these seedlings had completely green buds. In all the rest the buds were slightly tinged with pink, and bushes with red buds were not found.

4. FORM OF THE BUDS.

Most of these bushes had conical buds. About 30 per cent. had buds of an intermediate shape and on four bushes the buds were round. About one-third had buds with tightly arranged scales, in the rest they were loose and ragged. The buds protruded from the shoot in all the seedlings except ten, in which they were closely adpressed to it.

5. Season of Coming into Leaf.

Most of the seedlings were early in coming into leaf in the spring. A fairly large number were midseason, but only nine were late.

6. Flower Characters.

The flowers were usually rather small and often rather pale, but there were a few in which they were tinged with a bright pink. About half the bushes bore flowers in which the styles were on a level with the anthers and, on the other half, the styles were much above the level of the anthers. They were predominantly early in blossoming but, in about 30 per cent., the flowers were midseason. Late blossoming seedlings were not found. Long flowering racemes occurred on about 13 per cent., slightly more than half the bushes had racemes of medium length and, on the rest, they were definitely short. The racemes of all the seedlings were wavy, with the exception of nine, in which they were held stiffly outwards. Forty-one per cent. had an average of seven flowers per raceme, 51 per cent. an average of nine, while the remaining bushes had an average of eleven.

7. Fruit Characters.

Considerable variation in fruit size was found among these seedlings. About 30 per cent. bore large fruits at six years old, about 66 per cent. fruits of medium size, while, on the rest, the fruits were small. Fruits early in ripening occurred on about a quarter and those of about a quarter were quite late. On the rest, the fruits were midseason. Sweet flavoured fruits were found on about a third and the fruits of about 10 per cent. were definitely acid. On the rest, the fruits were not outstandingly sweet or acid. The fruits of approximately 65 per cent. had tough skins while those on the remaining bushes had tender skins.

8. Cropping.

At six years old, these seedlings gave an average of very slightly more than one pound of fruit per bush and, at seven years old, the average was 2·1 lb. Thirteen bushes bore more than twice the average, at six years old, and one bush gave more than three times the average. Several of these bushes had other valuable characters and have been thought worthy of retention for further trial.

DISCUSSION.

All the varieties of black currant appear to belong to the single species *Ribes nigrum* L. This species has been described in some detail by several

writers (1, 2), and these descriptions correspond very closely to those of the varieties of the black currant now in cultivation, from which they may possibly have been made. It may, perhaps, be doubted whether there is any purely specific form of *Ribes nigrum* in existence, apart from the numerous cultivated varieties. The writer has closely observed eight forms, received under the designation *Ribes nigrum* and now growing at East Malling, collected from localities as far apart as North Canada and Siberia. The differences between them are often greater than those between many recognized species of *Ribes*. The form which comes nearest to the published descriptions of *Ribes nigrum* was received from the Continent under that name. While it resembles the cultivated black currant fairly closely, it does not fall readily into any one of Hatton's groups and, indeed, appears to combine some of the characteristics of several of them. During the nine years that it has been growing at East Malling it has never set any appreciable quantity of fruit. Many of the other forms, received under the same name, bear little resemblance to the cultivated black currant except, perhaps, in the characteristic pungent odour. The very dwarfed growth and deeply laciniated, incised leaves of some suggest adaptation to conditions of considerable drought or cold while their fruits are unpalatable in the extreme.

For almost exactly a quarter of a century, East Malling has been studying the botany and breeding behaviour of black currant varieties. Such studies are an indispensable preliminary to any serious attempt to build up new and improved sorts. Hatton's original description and classification work showed that all the existing varieties had serious limitations in one respect or another and made it possible to envisage an ideal variety, possessing, in a single clone, good qualities assembled from a number of the present sorts. Such a variety should be vigorous and resistant to disease, should possess a long fruiting raceme, making picking easy and ensuring the maximum possible quantity of berries for a given size of bush, with large berries of good appearance. The blossoms should be borne profusely and be so formed as to ensure that there is little or no failure to set through imperfect pollination, and possessing, at the same time, qualities of frost resistance which will reduce loss from this source to a minimum. On the question of the season of ripening of the fruit, opinion might be somewhat divided, but probably many growers would prefer a variety maturing its crops early, thus enabling them to secure the better prices which of late years have been obtainable early in the season. The flavour of the fruit and the texture of its skin are characters of rather less importance. While there may be a slight prejudice in favour of varieties with sweet flavoured fruits, other things being equal, there is no evidence that such fruits command a better market than those of a more acid flavour ; in fact, in certain instances, the acid flavoured varieties have definitely been preferred for certain specific

purposes. Slightly greater importance might be attached, by most growers, to the texture of the skin of the fruit. Varieties with tender skinned fruits, of which Boskoop Giant is a good example, unless picked slightly under ripe, rarely travel as well as those with a tougher skin. There are certain other characters which make a considerable difference to the value of the fruit to the jam manufacturer or canner. The evenness with which the fruits ripen upon the bush and the ease with which the fruits can be "strigged" or removed from the raceme, may be mentioned.

One or other of almost all these qualities are possessed by the cultivated varieties. For example, Boskoop Giant has long fruiting racemes and large berries but, under conditions here, it is an unreliable cropper, very subject to "running off". Baldwin bears heavy crops in most seasons, but the bushes are small in size, the fruiting racemes of only medium length and the berries are not large. Goliath usually bears largish berries of a pleasant, sweet flavour, but the racemes are very short and, in most seasons, there is some "running off". In the varieties of the French group, the fruits are smallish, the racemes rather short and the fruits of an acid flavour, but some of the varieties in this group show resistance to Big Bud.

An extensive study of seedlings obtained from pollinating these varieties with their own pollen, showed, as was to have been expected, that little improvement could be effected by this means. Varieties in the French group gave rise to some seedlings of stunted growth and abnormal foliage which did not resemble the cultivated black currant at all, and suggested the possible hybrid origin of this group. But, in general, the seedlings from all these varieties reproduced the characters of their parents to a considerable extent and, in their general performance, showed little promise of any outstanding improvements.

Perhaps the most striking thing about the seedlings obtained from crossing these varieties was the comparatively limited range of variation which they have shown. While most of the characters of the cultivated varieties were reproduced in the seedlings, none of them showed any marked advance on the best existing sorts. For instance, no seedling was found with racemes longer than those of Boskoop Giant or with fruits larger than those of Goliath, although it is noteworthy that some seedlings with such characters as long racemes and large fruits occurred in families from crosses the parents of which did not possess these characters. But striking instances, such as have been reported in some fruits, in which seedlings showing a marked advance on cultivated sorts have arisen through the coming together of cumulative or complementary factors, were not found. This suggests that the breeder can look for little improvement in the expression of such characters as raceme length or fruit size as they are now found in such varieties as Boskoop Giant or Goliath, without going beyond the species *Ribes nigrum*. Quite evidently his chief line of

Table I.

Showing Number of Seedlings with the Four Characters, Long Racemes, Large Fruits, Sweet Flavour and Tough Skin, Singly or in Combination.

Parents.	Number of Seedlings.	Long racemes.	Large fruits.	Sweet flavour.	Tough skin.	Long racemes. Large fruits.	Long racemes. Sweet flavour.	Long racemes. Tough skin.	Large fruits. Sweet flavour.	Large fruits. Tough skin.	Sweet flavour. Tough skin.	Long racemes. Large fruits. Sweet flavour.	Long racemes. Large fruits. Tough skin.	Long racemes. Sweet flavour. Tough skin.	Large fruits. Sweet flavour. Tough skin.	Long racemes. Large fruits. Sweet flavour. Tough skin.
French Black and Seabrook's Black ..	63	5	8	3	39	1	1	3	1	3	0	0	0	0	0	0
French Black and Goliath	106	2	23	13	71	0	0	0	1	10	6	0	1	0	1	0
French Black and Boskoop Giant	133	20	15	12	63	2	2	5	1	4	0	1	2	1	0	0
French Black and Baldwin	111	4	15	11	73	0	0	4	3	7	4	0	0	0	1	0
Seabrook's Black and Goliath	104	1	12	4	70	0	0	1	1	4	1	0	0	0	1	0
Seabrook's Black and Boskoop Giant ..	109	12	21	7	50	6	0	4	1	5	0	0	1	0	0	0
Seabrook's Black and Baldwin	109	4	20	11	75	0	0	3	4	8	4	0	1	0	1	0
Goliath and Boskoop Giant	110	2	33	13	52	1	0	1	7	14	1	0	0	0	0	0
Goliath and Baldwin	111	2	34	32	63	0	0	0	5	16	10	1	0	0	2	0
Boskoop Giant and Baldwin	114	16	33	12	60	4	0	4	7	6	1	0	5	0	0	0

advance must be in the direction of combining within single varieties the best qualities already in existence.

As may be seen from Table I, in which the number of seedlings in these twenty families possessing the four fruit characters, long racemes, large berries, sweet flavoured fruits and tough skins are shown, it was a comparatively simple matter to obtain seedlings with any two of these characters. For example, long racemes and large berries were found in combination in 1 per cent. of all the seedlings, while long racemes and tough skins occurred in as many as 2 per cent. The combination in a single seedling of three of the four characters was a much rarer occurrence. Actually, the three characters, long racemes, large berries and sweet flavour were found together in less than two in a thousand seedlings. No seedling was found possessing the four characters in combination.

All the evidence so far collected seems to indicate that a number of characters in the black currant are controlled by a very simple genetic mechanism. This was to have been expected, since *Ribes nigrum* has been found to be diploid (4), without any marked irregularities in its cytological processes. Observation would suggest that such characters as season of leafing out in the spring, bud shape and the length of the racemes are controlled by factors obeying simple

TABLE II.

Showing Mendelian Inheritance in Three Characters.

	AaAa	Aaaa	aaaa
Season of coming into Leaf:			
Early (AaAa) × Midseason (Aaaa)	122	85	19
Theoretical Expectation	**113**	**85**	**28**
Early (AaAa) × Late (aaaa)	51	121	47
Theoretical Expectation	**55**	**110**	**55**
Midseason (Aaaa) × Late (aaaa)	0	221	232
Theoretical Expectation	**0**	**226**	**226**
Midseason (Aaaa) × Midseason (Aaaa)	11	74	24
Theoretical Expectation	**27**	**54**	**27**
Late (aaaa) × Late (aaaa)	0	0	64
Theoretical Expectation	**0**	**0**	**64**
Shape of the Buds:			
Conical (AaAa) × Conical (AaAa)	367	203	37
Theoretical Expectation	**342**	**228**	**38**
Conical (AaAa) × Round (aaaa)	123	287	75
Theoretical Expectation	**121**	**242**	**121**
Length of Fruiting Raceme:			
Long (AaAa) × Medium (Aaaa)	120	181	41
Theoretical Expectation	**171**	**128**	**43**
Medium (Aaaa) × Medium (Aaaa)	51	164	68
Theoretical Expectation	**71**	**142**	**71**
Medium (Aaaa) × Short (aaaa)	15	177	126
Theoretical Expectation	**0**	**159**	**159**
Long (AaAa) × Short (aaaa)	15	67	27
Theoretical Expectation	**27**	**54**	**27**

Mendelian laws of segregation and recombination. The inheritance of these characters is shown in detail in Table II. In all these cases pairs of factors, cumulative in their effect, appear to be involved. There are, however, a number of characters whose inheritance seems to be controlled by a more complex mechanism ; these include bud colour, flower structure and such fruit characters as flavour and the texture of the skin. The difficulty of finding any simple hypothesis upon which to explain the inheritance of these characters may, however, be due in part to the difficulty of assessing them with accuracy and to the modifying influence of environmental conditions, such as frosts. For instance, a very intimate relation has been shown to exist between the structure of the flower, i.e. whether the style is much above the level of the anthers or in the same plane with them, and the setting of the fruit. Where the style is on a level with the anthers, the chances of pollination have been shown to be much greater. But that other factors were operating in controlling the amount of fruit borne by these seedlings is suggested by the results set out in Table III, in which the weight of fruit borne by the seedlings with flowers

TABLE III.

Illustrating the Relation between the Position of the Style in the Flowers and the Amount of Fruit Borne.

Parents.	Position of Style.	
	Level with anthers.	Above anthers.
	Crop in lb.	per bush.
French Black × Seabrook's Black	0·64	0·75
French Black × Boskoop Giant	0·53	0·61
French Black × Goliath	1·19	0·90
French Black × Baldwin	0·84	0·92
Seabrook's Black × Boskoop Giant	0·63	0·60
Seabrook's Black × Goliath	0·72	0·86
Seabrook's Black × Baldwin	1·48	0·90
Goliath × Boskoop Giant	0·92	0·77
Goliath × Baldwin	1·46	1·05
Boskoop Giant × Baldwin	0·47	0·52
Average of all bushes	0·89	0·79

with styles above and on a level with the anthers, in their sixth year from the seed, are contrasted. Although, over all the seedlings, the average weight of fruit was slightly greater on the seedlings with level styles, in five out of the ten families, the average crop of the bushes with styles above the anthers was higher than that of bushes with level styles. It must be quite evident that such families will differ markedly in the number of flowers which they bear, in their natural fertility and in their susceptibility to frost or unseasonable

weather during flowering. Variations in the size of the bushes or in the length of the racemes, too, are likely to affect the quantity of fruit which can be carried. All these factors are likely to operate in masking the effect of a single character like the length of the style.

The present researches seem to point to the conclusion that larger families of certain of these crosses might have given seedlings with a greater number of desirable characters. It seems clear, as Table I shows, that certain families contain seedlings coming nearer to the ideal variety than others. Thus, the three characters, long fruiting trusses, large berries and sweet flavour, occurred in the same seedling only in the two families French Black × Boskoop Giant and Goliath × Baldwin. Had these families been ten times as large, the possibility of obtaining the three characters in combination with others equally valuable, would naturally have been much greater. It is evident, too, that any one of the nineteen seedlings, combining three of the four characters shown in Table I, is an advance in this respect on any variety at present in cultivation, since no one combines three out of the four of these characters. Crosses between these seedlings are, therefore, more likely to approach more nearly to the ideal variety than would first crosses between the present established varieties. Future work will lie in the direction of raising larger families from a few selected first crosses and of intercrossing the best of these seedlings.

For any great improvement in such characters as raceme length or fruit size beyond what is at present found in the best existing varieties, it seems fairly clear that hybridization with other related species, or some of the modern methods for facilitating the occurrence of mutations must be employed. The possibilities of either of these lines of work do not look particularly hopeful at present. Of the other species of *Ribes* which the writer has had an opportunity of examining, the most promising as a possible parent for new varieties of black currants appeared to be *Ribes longeracemosum,* a species from Eastern Tibet and Western China, which has long racemes from 12 to 18 inches long and large, black, good flavoured berries. Using the pollen of this species on the variety Baldwin, a number of seedlings were obtained and grown for several years at East Malling. There could be no doubt of their being true hybrids, because they resembled *Ribes longeracemosum* very closely, having distinctively dark, shiny, quite odourless leaves, with long, acutely tapering lobes and fruiting racemes up to a foot or more in length. The flowers had long, bifid styles much above the level of the anthers. But, during the seven years that these plants were kept under observation, they were always completely sterile. Attempts are now being made at the John Innes Horticultural Institution to induce polyploidy in these hybrids and thus to make them fruitful.

The possibility of obtaining an improvement in the seedling characters by inducing mutations by artificial means is also being explored. In 1931 and

1932, the late Mr. L. H. Stone, of the John Innes Horticultural Institution, subjected seed, pollen and ovules of the black currant varieties, Baldwin and Boskoop Giant to treatment with X-rays for periods of varying duration. For ovules and pollen mother cells, the intensity was 64 kilovolts, 5 milliamps tube current, unscreened radiation at 30 cm. target distance and the duration was 3, 6, 9 and 15 minutes, on various dates between December 2nd, 1931, and March 24th, 1932, by which latter date the flowers were becoming mature. Seeds from self pollinated fruits of Baldwin were subjected to a voltage of 90 kilovolts, tube current 5 milliamps, unfiltered radiation at a distance of 25 cm. for periods increasing from 2 to 32 minutes, by additions of 2 minutes, on a single day in the autumn of 1931. The treated ovules were fertilized with treated pollen, and X-rayed pollen was applied to untreated flowers. After six years of careful observation of nearly two thousand seedlings, raised from such treated seeds and fruits, no perceptible effect of these treatments could be seen. The bushes were perfectly normal in appearance and quite fruitful. Seedlings have been raised from a number of these bushes and observations will be continued upon a further generation.

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The photographs were taken for the writer by Miss K. E. Cornford.

SUMMARY.

An account is given of observations extending over seven years, on twenty families from crosses between the main varieties of black currants.

The inheritance of the following characters is dealt with: *Leaves:* shape, colour, pose, depth of basal sinus, colour of petioles. *Vigour* and *Season of Coming into Leaf*. *Flowers:* colour, season of blossoming, length of styles. *Racemes:* length and pose. *Fruits:* size, flavour, texture of skin, season of ripening. *Cropping*.

The characteristics of the ideal black currant variety are discussed and it is shown that while each of the existing commercial varieties has a few desirable characters, no one of them comes near to realizing the ideal completely.

It is thought that the inheritance of certain characters such as season of coming into leaf, shape of the buds and length of the flowering racemes, follow simple Mendelian laws, as was to have been expected, since the black currant is a diploid. Other characters are thought to be much more complex in their mode of inheritance.

The lines along which the work is developing are indicated. It is suggested that larger families from first crosses between certain varieties and crosses between the most promising of the seedlings are likely to give the best results. The possibilities of hybridization between related species of *Ribes* are discussed. Some seedlings from a cross between the black currant and a Chinese species, *Ribes longeracemosum*, are described. These hybrids have been quite sterile during seven years. Attempts to induce mutations in black currant seedlings by treating seeds, ovules and pollen with X-rays have so far proved completely ineffectual.

REFERENCES.

(1) *Berger, A.* A Taxonomic Review of the Currants and Gooseberries. New York State Agric. Exp. Sta. Tech. Bull., 109. 1924.

(2) *Card, F. W.* Bush Fruits, 1907, 4th Edition.

(3) *Hatton, R. G.* Black Currant Varieties. A Method of Classification. Journ. Pom. & Hort. Sci., 1920, **1**, 65.

(4) *Meurman, O.* Cytological Studies of the Genus *Ribes* L. Hereditas, 1928, **11**, 269.

(5) *Tydeman, H. M.* Some Results of Experiments in Breeding Black Currants. 1. The Self Pollinated Families. Journ. Pom. & Hort. Sci., 1930, **8**, 106.

(6) *Wellington, R., Hatton, R. G.* and *Amos, J.* The "Running Off" of Black Currants. Journ. Pom. & Hort. Sci., 1921, **2**, 160.

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FIG. 1. Illustrating differences in the depth of the basal leaf sinus.
Left to right, deep, intermediate, shallow.

FIG. 2. Illustrating differences in the pose of the leaves.
Left, blades downturned. Right, blades curved over the petioles.

FIG. 3. Illustrating differences in the season of ripening of the fruits. Shoots collected on the same day from the same family.
Left, ripe fruits. Centre, half ripe fruits. Right, very unripe fruits.

FIG. 4. Illustrating differences in the length of the fruiting racemes and size of fruits. Shoots collected from the same family.
Left, long racemes and medium sized fruits. Centre, racemes of medium length and small fruits. Right, short racemes and large fruits.

THE RELATIVE INFLUENCE OF ROOTSTOCK AND OF AN INTERMEDIATE PIECE OF STOCK STEM IN SOME DOUBLE-GRAFTED APPLE TREES

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INTRODUCTION.

THE rootstock in this country normally includes a piece of stock stem as well as a root system and it is in this sense that the term will be used here. It is impossible to know *a priori* whether the influence of such a rootstock on a scion is due solely to the root system, solely to the piece of stock stem, or in part to each. Knowledge on this matter would be useful ; for, if all, or part, of the influence is due to the stem, it should be possible to regulate the intensity of the influence of a stock by using longer or shorter pieces of stock stem, or even to combine the influences of two or more stocks by introducing lengths of their respective stems into the trunk.

Some years ago, Knight (6) published a preliminary report on some experiments with double-worked apple trees, planned to give information on the relative parts played by roots and stems in stock influence. Knight followed up this report by another (7) in which he described the performance of the same trees to the end of their sixth year, when they were lifted and weighed.

The trees used in that experiment were composed of three parts, united by graft-unions ; (a) a scion variety, (b) an "intermediate" piece of stock stem, and (c) a rootstock made up, as described above, of a root system and a short piece of stem to which it was attached. The scion was the same in each case—Lane's Prince Albert—whilst the intermediates and rootstocks were of the Malling stock varieties No. I (vigorous) and No. IX (dwarfing), all four possible combinations of the two stocks as intermediate and rootstocks being used, i.e. A (I/I), B (IX/I), C (I/IX) and D (IX/IX). The effect of stock stem was examined by the use of additional pieces of stem rather than by the reduction or elimination of the piece normally present ; this was done, as Knight pointed out, because the inclusion of a short length of stem of the same variety as the roots, though undesirable, was preferable to the confusion that would result from scion rooting, which might occur if the intermediates were grafted direct on to pieces of root.

The total wood growth, the number of fruit buds and the fruit buds per metre wood growth were determined on each type of tree.

The preliminary results (6) indicated "that whilst the root system of the stock is probably the dominating factor in the influence of the stock upon scion, yet at the same time, the stem of the stock also plays a part which may be considerable". The subsequent behaviour of these trees, described in the second paper (7), provided additional evidence "that the part played by the stock stem in influencing the scion is subsidiary to that played by the root system".

Knight pointed out that in these trees it was impossible "to differentiate completely the influence of the roots and that of the stem", because (a) the stem below the scion was not composed solely of the intermediate stem piece but, in series B, for example, included also a short length of No. I stem (to which the roots were attached) as well as the intermediate piece of No. IX stem; (b) the trees were double-worked in two operations, separated by a season, the intermediate being whip-grafted, as low as possible, on to the stem of the root system one year and allowed to function for a season as a scion before the scion variety (Lane's) was grafted on the top. "Thus for one season the root systems of series A were under the influence of vigorous scions," No. I, "whilst those of series B were under the influence of dwarfing scions," No. IX. "It is known that a root system may be modified to some extent by the nature of the scion grown upon it, and therefore it cannot be assumed that the two series were comparable, as regards root systems, at the time of grafting the second scions; (c) "It must be realized that these experiments take no account of any influence which might accrue from the union itself, as distinct from both stem and root of the stock." For instance, "Series A possess a Lane's/I and I/I union, whilst series B have a Lane's/IX and a IX/I union"; (d) "Each series consisted of six trees only and conclusions must therefore be regarded as only tentative."

With regard to (b) and (d), Knight mentioned that a larger series of trees was being prepared "in which the difficulty of the influence of the first scion on the rootstock will be overcome by the use of 'budded grafts'". The trees used in the experiment described in the present paper were not the "budded grafts" mentioned by Knight; their performance will be described elsewhere. They were a series of trees double-grafted at one operation.

MATERIAL.

Types of Tree.

The trees used were double-grafted and made up of three parts: *Rootstock* (below lower union), *Intermediate* (between unions), and *Scion* (above upper union). The scion was always Stirling Castle, the stock and intermediate were either No. IX or No. XII which were combined as stock and intermediate in all four possible ways. There were thus four types of tree:—

	Scion.	*Intermediate.*	*Rootstock.*
A (XII/XII)	Stirling Castle	No. XII	No. XII
B (IX/XII)	,, ,,	No. IX	No. XII
C (XII/IX)	,, ,,	No. XII	No. IX
D (IX/IX)	,, ,,	No. IX	No. IX

Method of Working.

The trees were prepared by the Propagation Section of this Station as follows: Stocks were removed from the stoolbeds in the winter of 1929-30 and planted in nursery rows. In the winter of 1931-32, after they had completed two years of growth, the top of each was cut off a few inches above ground level and the lower portion of the severed top was then grafted on to the same stump, or on to the stump of another rootstock. A piece of the scion variety (Stirling Castle) was grafted on to the top of each at the same time. The object of doing both operations at one time was to avoid the possible after-effects that might occur if two unions were made in successive years, so that the stock used as intermediate would function also for one year as scion. Care was taken to ensure that the length of these grafts was approximately the same in all trees.

The trees were left in position in the nursery rows for the growing season of 1932, one scion shoot being allowed to grow out from each to form a maiden tree. In the winter of 1932-33 they were lifted, weighed and replanted.

Plan of Planting.

The trees were replanted in four rows of 38 each, trees 1 and 38 being buffers. The 144 experimental trees—36 in each row—were arranged in nine 4×4 squares. Eight of these were Latin squares, the other, owing to a shortage of C trees, was made up of 5 A's, 4 B's, 3 C's and 4 D's. One square had to be broken into when a new packing shed was built, and casualties occurred, mainly through canker, in some other squares. At the end of the experiment, data were available from 126 trees made up of 31 A's, 30 B's, 32 C's and 33 D's. Sixty-four of these trees, sixteen of each combination, were from complete Latin squares; the remainder from broken squares. The conclusions drawn will be based mainly on the full number of trees, but in certain cases, where a complete analysis of variance seems desirable, only the values from complete squares will be used, as these lend themselves more readily to such an analysis.

Routine Treatment of Trees.

The trees were all manured alike and, as far as possible, pruned alike. They were deblossomed each year before fruit set. Severe suckering occurred on all types except A (XII/XII); these suckers were removed.

The following measurements or counts were made on each tree every year:—

(1) Total "wood growth" or length of new shoots; this was done before pruning.

(2) Number of fruit buds formed in the summer—estimated by counting the blossom trusses the following spring. Those on the prunings were included. The "spur", "axillary" and "terminal" blossom trusses were counted separately, but for the purposes of this paper they have been summed together.

(3) The diameter of the trunk was determined at three positions: (a) on *stock* stem (below lower union), (b) on *intermediate* stem (between the unions) and (c) on 1932 wood of *scion* (above upper union). To ensure that the measurements should be made at the same places each year, paint marks were made on the trees in 1932 and renewed in subsequent years. These can be clearly seen in Fig. 2. The measurement was made in two directions, N.S. and E.W. in each case and the mean value used. The values for the cross sections for these mean diameters were found from Castle's Tables.

Final Measurements.

The trees were dug up with reasonable care in December 1937, and, though not excavated by the trench method, it is probable that most of the roots were recovered. The trees were photographed and certain typical examples of each combination were varnished and set aside as permanent specimens.

Each of the remaining trees was weighed three times; as a whole, after the removal of the branches, and after the removal of the roots. Weights of branches and of roots were got by the differences.

The trunks included the pruned remains of the 1932 (maiden) scion wood and the underground portion of the original rootstock stem. The branches consisted of the unpruned 1937 shoots and the pruned remains of those formed in the seasons 1933 to 1936 inclusive. Spurs were included with branch weights.

The trunks were measured to determine the approximate lengths of the stems of the rootstock, intermediate and scion, and of the upper and lower unions, also the position of the paint marks where the diameters had been measured.

Statistical Analysis.

As the differences are for the most part large and the errors, where worked out, are mostly small, it has not been considered necessary to go to the labour of working out all the standard errors throughout as a matter of routine. They have been worked out, however, for the more important data.

Certain of the data from the complete Latin squares have been examined statistically by the method of analysis of variance.

RESULTS.

General Character and Size of Tree.

Two typical trees of each combination, A, B, C and D, are shown in Fig. 1, and the trunks of three typical trees of each kind are shown on a larger scale in Fig. 2, A, B, C and D. In each case the four photographs are on approximately the same scale.

The relative sizes of the trees show up well in Fig. 1. The trees of type A (XII/XII) were much the largest, and those of type D (IX/IX) the smallest. The B (IX/XII) trees were intermediate in size, whilst the C (XII/IX) trees were only slightly larger than the D (IX/IX) trees.

Root Systems and Suckers.

The intermediate piece of stem had no perceptible effect on the morphology of the root systems ; these were typical of No. XII in A and B, and of No. IX in C and D. No suckering occurred in trees of type A, but it was rather pronounced in the other three types of tree.

Character of the Graft Unions.

The character of the unions can be seen in Fig. 2. The upper (scion/intermediate) union is between the top and middle paint marks, the lower (intermediate/rootstock) union between the middle and bottom paint marks.

There was a pronounced swelling at and above the upper union in trees of combination B and D, where the scion variety, Stirling Castle, was worked on to intermediate stem pieces of No. IX. A pronounced swelling of a rather different shape occurred at the lower union in combination C, where the intermediate stem piece of No. XII was worked on to a rootstock of No. IX. There was no trace of a swelling at the upper union where Stirling Castle was grafted on to intermediates of No. XII (A and C), or at the lower unions in A and D, where intermediate and rootstock were both of the same kind. Although no apparent swelling formed at the lower union in B, where a piece of No. IX stem had been worked on to No. XII, the whole piece of No. IX stem had grown to a larger size than that of the No. XII stem below it, and this growth may have been analogous to the formation of a swelling.

Length of Stem Pieces and Graft Unions.

The lengths of the scion, intermediate and stock components of the tree trunks were not recorded at the time of planting, but were determined at the time of lifting. The length of a component in a grafted tree cannot be measured exactly owing to the overlap at the unions where two components taper and

fit into one another. In making the measurements, therefore, the lengths of the graft unions were determined as well as those of the stock stem (below the lower union), the intermediate (between the unions), and the scion (above the upper union). The mean values of these lengths are given in Table I. The mean lengths of the component parts of the trunks were much the same in the four kinds of tree. The rootstock stem, most of which was underground, was slightly longer in trees on No. IX rootstock (C and D) than in those on No. XII rootstock (A and B), but the difference was not great enough to be important.

TABLE I.

Length of Trunk and its Component Parts, after Removal of the Branches and Roots.

	A.	B.	C.	D.
Scion		Stirling Castle.		
Intermediate Stem Piece ..	No. XII.	No. IX.	No. XII.	No. IX.
Rootstock	No. XII.	No. XII.	No. IX.	No. IX.
	cm.	cm.	cm.	cm.
SCION *Maiden Stem*, length (after pruning) of stem made in year after grafting	20·6	19·4	19·4	18·8
SCION *Graft*, length of portion of graft above upper union ..	5·8	7·6	7·0	6·9
Upper Union (overlap of scion and intermediate)	6·1	5·7	5·5	6·0
INTERMEDIATE length between unions	9·3	9·1	9·3	8·4
Standard Error	**·250**	**·225**	**·279**	**·190**
Lower Union (overlap of intermediate and rootstock) ..	5·9	5·5	6·1	6·0
ROOTSTOCK (including portion below ground to which the roots were attached) ..	20·8	22·5	25·8	26·7
Total Length of Trunk	68·5	69·8	73·1	72·8

The standard errors have been worked out for the mean lengths of the intermediate (between the unions) and are given in Table I. The mean length of the intermediate was slightly less in the D (IX/IX) trees than in the others, but the difference was less than 1·0 cm. and, though significant, is not likely to have had any important effect on the behaviour of the trees.

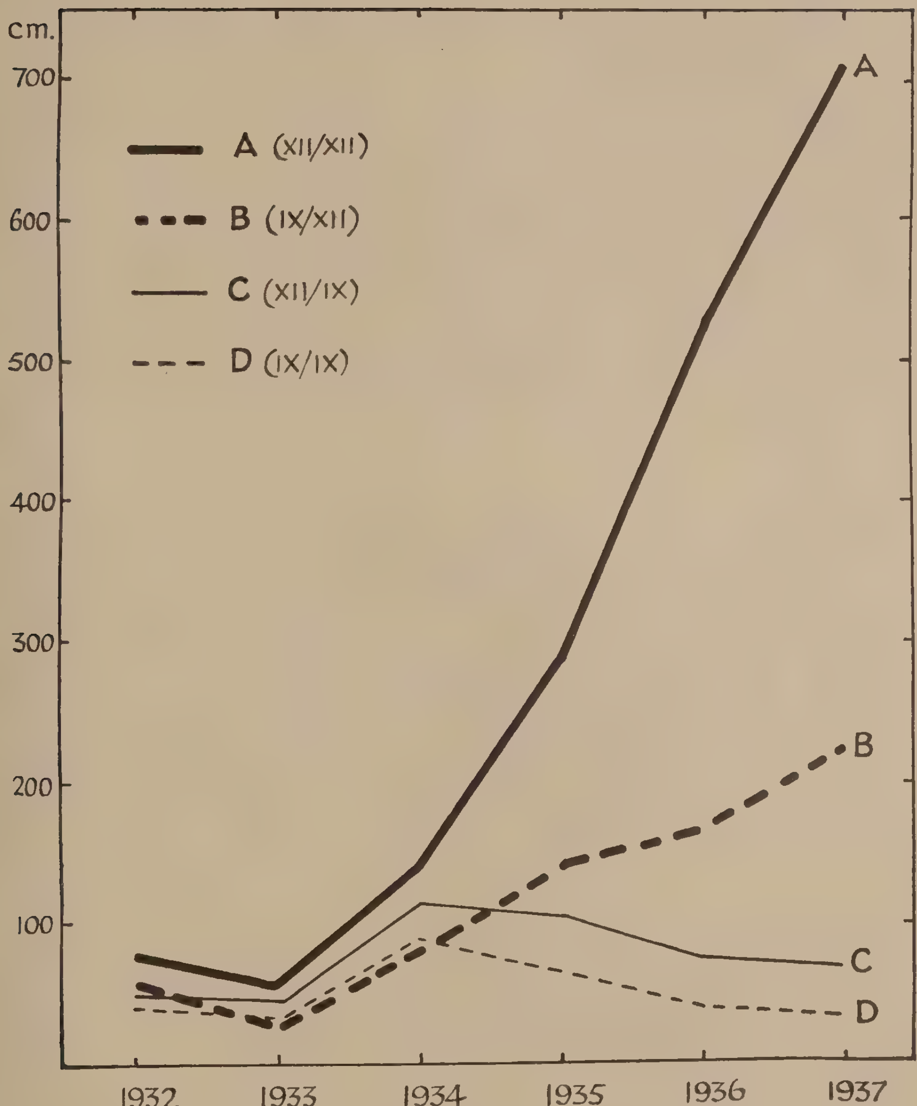

FIG. 3.
Wood Growth.
Mean values for summed length of new shoots formed each year on the four kinds of tree.

Annual Wood Growth.

The mean values for the summed lengths of new shoots made per tree each year are plotted in Fig. 3.

All trees made poor growth in 1933, the year after they were transplanted, but made better growth in 1934. In 1935, 1936 and 1937, the trees on roots of No. XII tended to increase their wood growth each year (A and B), whilst the trees on roots of No. IX (C and D) tended to decrease it. The values for A (XII/XII) were consistently higher than those for B (IX/XII), and those for C (XII/IX) consistently higher than those for D (IX/IX). But the values for B (IX/XII) resemble those for A (XII/XII) more closely than those for D (IX/IX), and the values for C (XII/IX) resemble those for D (IX/IX) more closely than those for A, suggesting that though the stock contributing the intermediate had a considerable effect on the wood growth of the scion, it had less effect than the stock contributing the root system.

Fruit Buds.

The mean number of fruit buds formed each summer—and counted as blossom trusses the next spring—are plotted in Fig. 4.

The trees on rootstocks of No. XII have consistently formed fewer fruit buds than those on rootstocks of No. IX, but at first sight it would appear that the effect of the intermediate stem piece has not been consistent. When the rootstock was No. XII, trees with intermediate No. IX (B) produced more fruit buds than those with an intermediate of No. XII (A), but where the rootstock was No. IX (C and D), the higher number was associated with the No. XII intermediate (C). The inconsistency vanishes, however, when the number of fruit buds is adjusted for size of tree.

Fruit Buds per Metre Wood Growth.

The values for fruit buds plotted in Fig. 4 were divided by the corresponding values for wood growth plotted in Fig. 3, and the resulting quotients have been plotted in Fig. 5. The values for trees with an intermediate of No. IX (B and D) are now consistently higher than those with an intermediate of No. XII (A and C).

The values in this graph are only approximations since they are the ratios of mean values and not the mean values of the ratios for individual trees. The latter, however, could not be worked out, because the denominator (wood growth) for some trees was occasionally nil owing to there being no shoots of over 5 cm. in length, whilst the numerator (fruit buds) was appreciable, owing to the presence of the spurs.

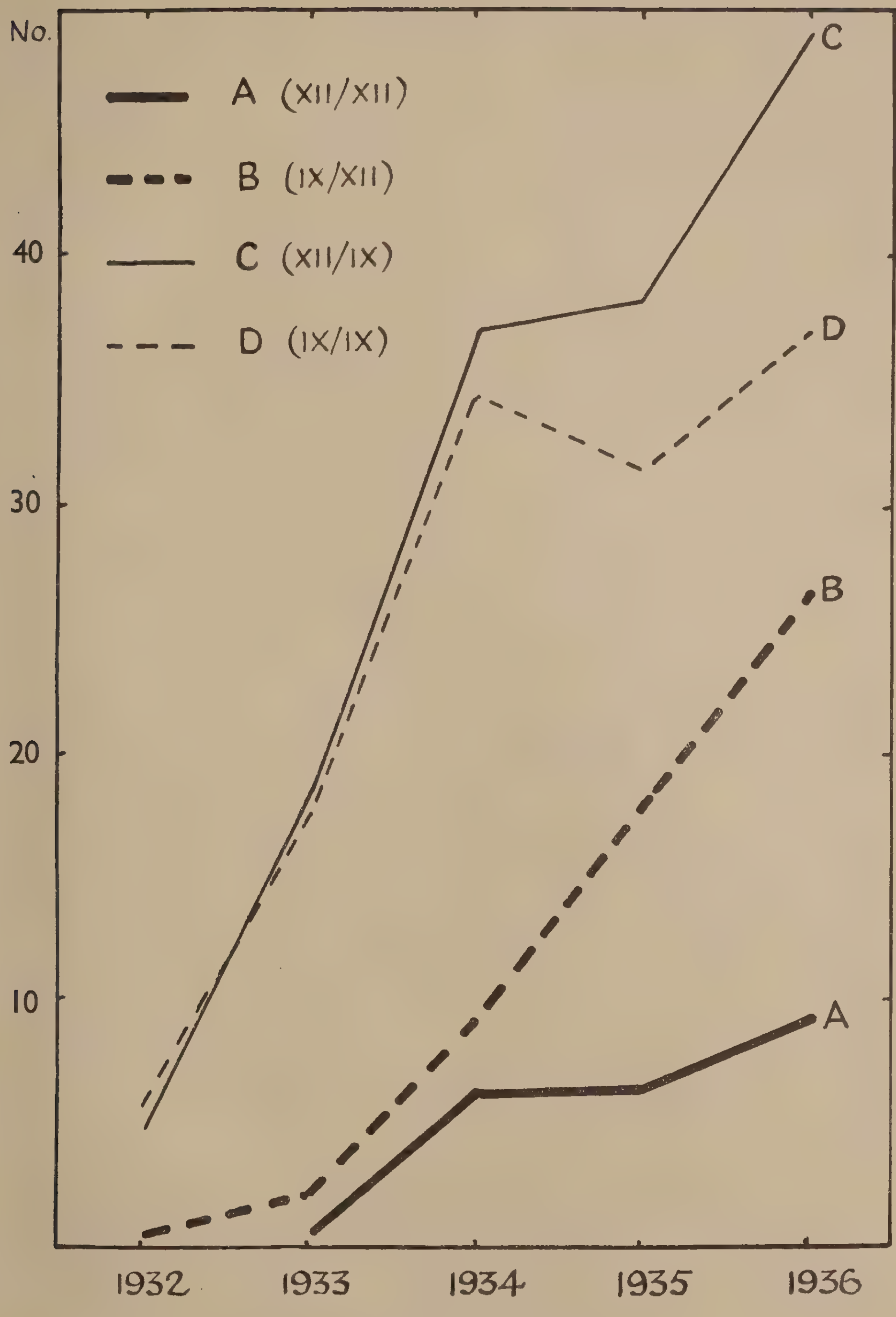

FIG. 4.

Number of Fruit Buds.

Mean number of fruit buds formed each year on the four kinds of tree.

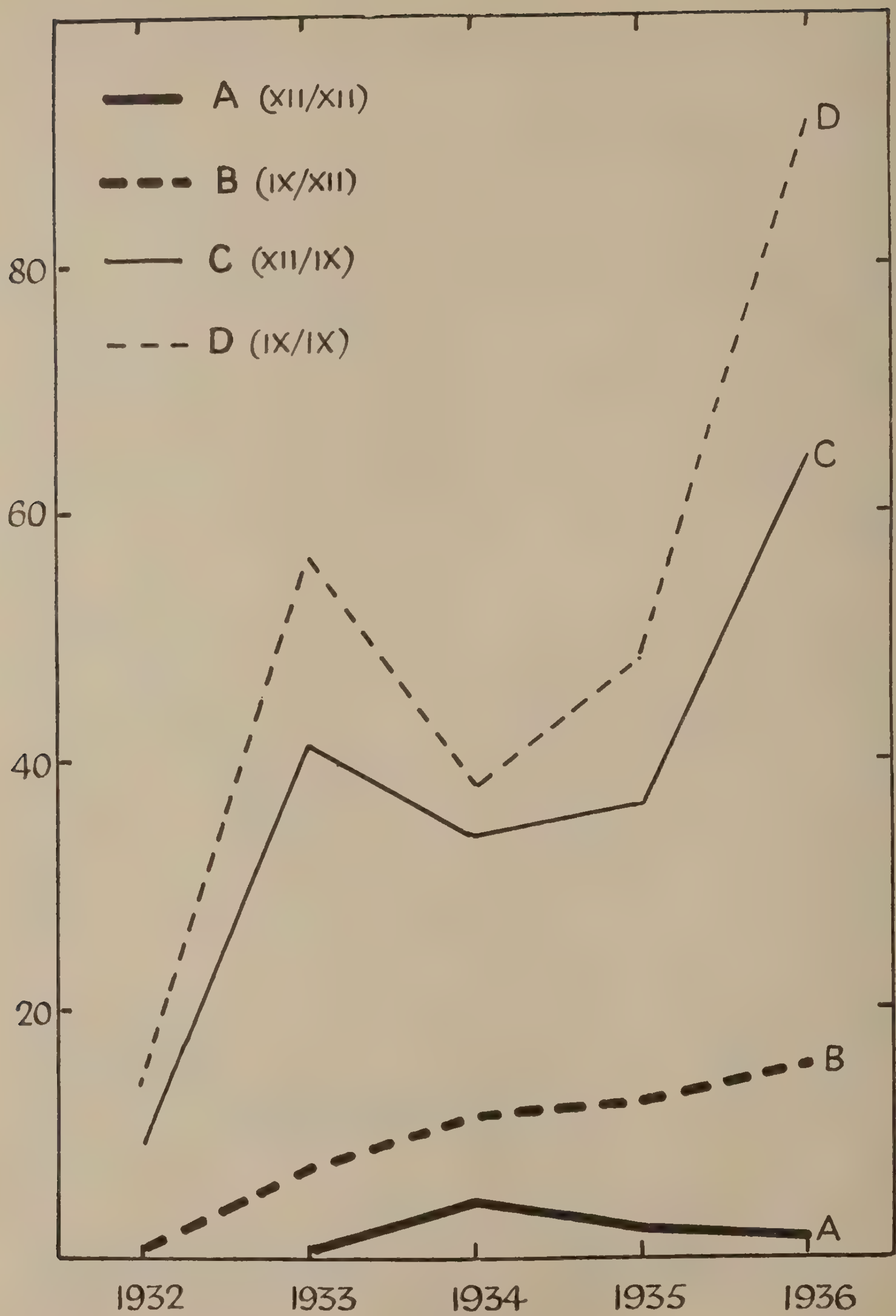

FIG. 5.

Number of Fruit Buds per metre wood.

Mean number of fruit buds per metre wood on the four kinds of tree, obtained by dividing the values plotted in Fig. 4 by those plotted in Fig. 5.

SUMMED WOOD GROWTH.

The values for wood growth for all years up to the end of 1936, and the end of 1937, have been summed for each tree, and the mean values and their standard errors are given in Table II. The values for the trees from (a) complete squares and from (b) broken squares were worked out separately, to determine whether there was any appreciable difference between the two sets of trees; the differences were small and in no case significant.

The differences between the mean values for the four kinds of tree, A, B, C and D, are large and, in most cases, thoroughly significant. The only difference whose significance is in doubt is that between B and C for wood growth to the end of 1936; here the difference is not significant if the trees from complete squares or from broken squares are taken separately, but is just significant if the mean values and standard errors are based on the full number of trees.

These results show that the summed wood growth was greater:—

(1) In trees on No. XII rootstock than in those on No. IX, whether the intermediate was No. XII, as in A and C, or No. IX as in B and D.

(2) In trees on No. XII intermediates than in those on No. IX intermediates, whether the rootstocks were No. XII as in A and B, or No. IX as in C and D.

(3) In trees on No. XII rootstock with an intermediate of No. IX than in those on No. IX rootstock with an intermediate of No. XII (B and C), the difference being just significant by the end of 1936 and thoroughly significant by the end of 1937.

A full analysis of variance was carried out on the values for summed wood growth to the end of 1936 in the trees from complete squares. The resulting standard errors, found from pooled variances after elimination of the parts due to "row", "position in row" and "square", are given in Table III, together with the values for "significant differences" calculated from them. These are the differences that may be expected to occur by chance only once in twenty occasions ($P = \cdot 05$) or only once in one hundred occasions ($P = \cdot 01$). The value for the standard error for the mean of a group of sixteen trees (64·25) is higher than those found for B, C and D in Table II, though less than that for A. This is due to the high value of the contribution of the A trees to the pooled variances. As a result the value for significance (182) is greater than that found for the difference between the mean values for C and D trees ($380 \cdot 5 - 260 \cdot 1 = 120 \cdot 4$), though the significance of this difference has already been proved by a straightforward "t" test.

At the bottom of Table III the comparison is made between the mean values for groups of thirty-two trees: (a) on No. XII (A+B) and on No. IX (C+D) rootstocks, (b) on No. XII (A+C) and on No. IX (B+D) intermediates

TABLE II.

Mean Values of Summed Wood Growth, Summed Fruit Buds and their Ratios. Values for trees from (a) Complete Squares and (b) Broken Squares given separately.

		Wood Growth Summed to end of 1936.				Wood Growth Summed to end of 1937.				Fruit Buds Summed to end of 1936.		Fruit Buds per metre Wood.	
		Mean.	S.E.	Mean.	S.E.	Mean.	S.E.	Mean.	S.E.	Mean.	Mean.	Mean.	Mean.
A. XII/XII	(a)	1099·0	**137·80**			1782·5	**214·01**			23·9		2·4	
				1078·7	**95·64**			1788·8	**148·89**		21·9		2·2
	(b)	1057·0	**136·89**			1795·5	**214·06**			19·7		2·0	
B. IX/XII	(a)	487·4	**43·25**			730·1	**69·10**			53·1		11·4	
				473·6	**28·86**			699·5	**46·20**		55·5		12·2
	(b)	457·9	**41·88**			664·6	**61·09**			58·4		13·0	
C. XII/IX	(a)	380·5	**35·25**			434·0	**42·88**			144·3		39·6	
				388·6	**20·68**			463·6	**27·16**		147·0		39·1
	(b)	396·6	**22·71**			493·1	**33·06**			149·8		38·6	
D. IX/IX	(a)	260·1	**19·66**			290·4	**23·91**			125·6		50·0	
				267·1	**15·36**			299·0	**17·40**		125·9		49·2
	(b)	273·7	**23·87**			307·1	**25·75**			126·1		48·4	

and (c) those in which both rootstock and intermediate were of the same stock variety (A + D), and those in which they were of different varieties (B and C). In each case the differences are thoroughly significant. The summed wood growth was greater on No. XII than on No. IX rootstocks, on No. XII than on No. IX intermediates and when rootstock and intermediate were of the same than when they were of different varieties.

TABLE III.

Mean Values, Standard Errors and Significant Differences for Summed Wood Growth to end of 1936, and to end of 1937, also for Summed Fruit Buds to end of 1936 and for the Ratio (Summed Fruit Buds per Metre Summed Wood) to end of 1936. The Values are for the 64 Trees from Complete Squares.

	Summed Wood Growth.	Summed Fruit Buds.	Fruit Buds per metre Wood Growth.
A. (XII/XII) ..	1099·0	23·94	2·4
B. (IX/XII) ..	487·4	53·06	11·4
C. (XII/IX) ..	380·5	144·25	39·6
D. (IX/IX) ..	260·1	125·63	50·0
Mean	556·77	86·72	25·86
S.E. (16 trees) ..	64·25	4·85	1·32
(32 trees) ..	45·43	3·43	·93
A − B	+ **611·6**	− **29·12**	− **9·0**
A − C	+ **718·5**	− **120·31**	− **37·2**
A − D	+ **838·9**	− **101·69**	− **47·6**
B − C	+ 106·9	− **91·19**	− **28·2**
B − D	+ **227·3**	− **72·57**	− **38·6**
C − D	+ 120·4	+ **18·63**	− **10·4**
Significant P·05 ..	181·7	13·72	3·7
Difference P·01 ..	245·3	18·53	5·1
Rootstock	+ **472·9**	− **96·44**	− **37·9**
Intermediate	+ **366·0**	− 5·25	− **9·7**
Interaction	+ **245·6**	− **23·83**	− ·7
Significant P·05 ..	128·5	9·7	2·6
Difference P·01 ..	173·4	13·1	3·6

SUMMED FRUIT BUDS.

The fruit buds formed each year to the end of 1936 were summed for each tree and the mean values are given in Table II. The corresponding values for trees from (a) complete and (b) broken squares agree so well that it seemed sufficient to base the analysis on the set from complete squares.

The results of the analysis are given in Table III. The differences between the mean values for the four kinds of tree, A, B, C and D, are all thoroughly significant and confirm the conclusions drawn from inspection of Fig. 4.

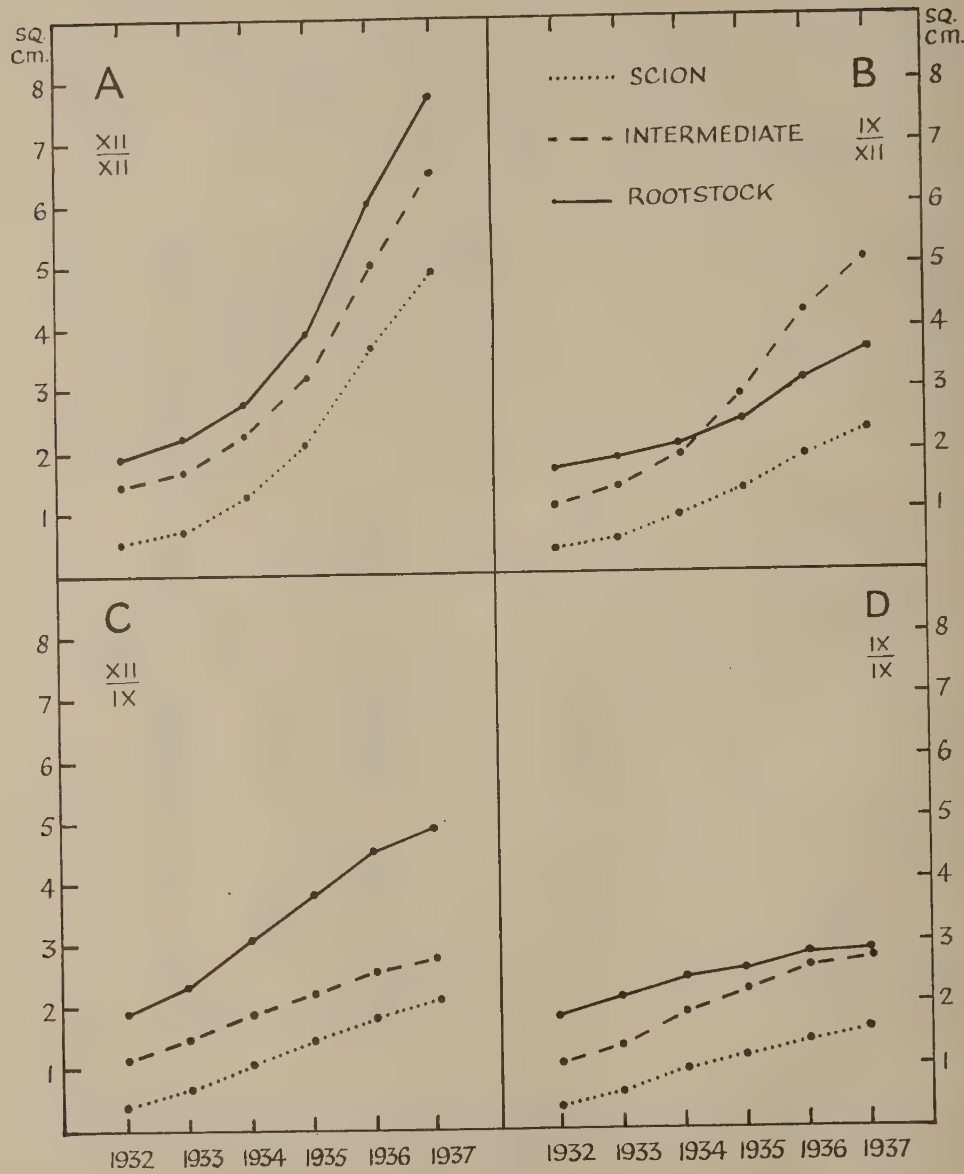

FIG. 6.

Cross section of Scion, Intermediate Stem Piece and Rootstock portions of trunk.

TABLE IV.

Cross Section of Scion, Intermediate Stem Piece and Rootstock Portions of Trunk at Time of Lifting in 1937. Values for Trees from (a) Complete Squares and (b) Broken Squares given separately.

		Scion.				Intermediate.				Rootstock.			
		Mean.	S.E.	Mean.	S.E.	Mean.	S.E.	Mean.	S.E.	Mean.	S.E.	Mean.	S.E.
A. XII/XII	(*a*)	5·011	·5821	4·950	·3747	6·684	·6871	6·578	·4482	7·681	·8134	7·714	·5325
	(*b*)	4·885	·4837			6·464	·5901			7·748	·7068		
B. IX/XII	(*a*)	2·393	·1581	2·328	·1096	5·293	·4142	5·050	·2964	3·786	·2036	3·615	·1636
	(*b*)	2·254	·1537			4·773	·2603			3·419	·2604		
C. XII/IX	(*a*)	2·035	·1440	2·089	·0877	2·696	·1538	2·777	·1030	4·739	·3668	4·885	·2161
	(*b*)	2·103	·1044			2·858	·1390			5·030	·2360		
D. IX/IX	(*a*)	1·545	·0655	1·552	·0522	2·780	·1543	2·763	·1121	2·903	·1366	2·885	·0941
	(*b*)	1·559	·0824			2·747	·1667			2·868	·1335		

Summed Fruit Buds per Metre Summed Wood Growth.

The values for summed fruit buds to the end of 1936 were divided by the corresponding values for summed wood growth for the same period ; these ratios could be, and were, worked out for the individual trees, as the value for summed wood growth was never nil. The mean value for trees from (a) complete and (b) broken squares are given separately in Table II, together with the general means based on the full number of trees. Agreement between the corresponding values for complete and broken squares was again very close and the analysis of variance was based on those for complete squares alone.

The results of the analysis are given in the last column of Table III. All the differences between the mean values for groups of sixteen trees are thoroughly significant and all are negative. The effect of rootstock was about the same whether the intermediate was No. XII (A – C) or No. IX (B – D), the values being 37·2 and 38·6 respectively. Similarly the effect of intermediate was about the same whether the rootstock was No. XII (A – B) or No. IX, being 9·0 and 10·4 respectively. The trees with No. XII rootstocks and No. IX intermediates had far fewer fruit buds per metre wood than those with No. IX rootstocks and No. XII intermediates (B – C). The effect of rootstock was greater than that of the intermediate as is shown by the values at the bottom of the Table, where the trees are compared in groups of thirty-two ; the difference between the mean values for the rootstocks was 37·9, that between the intermediates only 9·7. Finally, there was no significant difference between trees in which rootstock and intermediate both belonged to the same stock variety (A + D) and those in which they belonged to different varieties (B + C), indicating that effects of rootstock and intermediate were additive.

Area of Cross Section of Trunk.

The mean values for the cross section of scion, intermediate and rootstock portions of the trunk are plotted in Fig. 6 ; they are based on the full number of trees. The mean values at the time of lifting in 1937 are given separately for trees from (a) complete and (b) broken squares in Table IV, together with their standard errors. An analysis of variance was carried out on the values from complete squares and the resulting standard errors and "significant differences" are given in Table V.

The cross sections for trees of combination A (XII / XII) increased much more rapidly than those for the other combinations and attained significantly higher values (Table VI). The difference between the final values of B and C was significant only in the case of the intermediate, that between those for C and D only in the case of stock, whilst those between the final values for B and D were significant in the case of scion and intermediate but not for stock. Thus,

TABLE V.

Mean Values, Standard Errors and Significant Differences for the Cross Section of Scion, Intermediate Stem Piece and Rootstock Portions of Trunk at the Time of Lifting at the end of 1937; also for the Rates of Growth, expressed as the differences between the Natural Logarithms of the Weights at the Time of Planting (W_0) and at Lifting (W_1).

The Values are for the 64 Trees from Complete Squares.

		Area of cross section, 1937.			$(\log_e W_1 - \log_e W_0) \times 100$
		Scion.	Interm.	Stock.	
	Mean	2·75	4·36	4·78	147·85
S.E.	Group of 16	·211	·372	·436	7·24
S.E.	Group of 32	·150	·263	·308	5·12
XII/XII-IX/XII	A−B	+2·62	+1·39	+3·89	+36·0
XII/XII-XII/IX	A−C	+2·97	+3·99	+2·94	+70·2
XII/XII-IX/IX	A−D	+3·46	+3·90	+4·78	+79·2
IX/XII-XII/IX	B−C	+ ·35	+2·60	− ·95	+34·2
IX/XII-IX/IX	B−D	+ ·84	+2·51	+ ·89	+43·2
XII/IX-IX/IX	C−D	+ ·49	− ·08	+1·84	+ 9·0
Significant	P ·05	·60	1·05	1·23	20·4
Difference	P ·01	·74	1·42	1·66	27·7
Rootstock	(A+B)—(C+D)	+1·91	+3·25	+1·92	+56·7
Intermediate	(A+C)—(B+D)	+1·56	+ ·65	+2·87	+22·5
Interaction	(A+D)—(B+C)	+1·07	+ ·74	+1·03	+13·5
Significant	P ·05	·42	·74	·87	14·5
Difference	P ·01	·57	1·00	1·18	19·5

TABLE VI

Fresh Weights at Planting and at Lifting. Relative Branch and Root Weights and Relative Growth Weight.

The Values are for the 64 Trees from Complete Squares.

	A. XII/XII	B. IX/XII	C. XII/IX	D. IX/IX	Mean.
Fresh Weight at Planting ..	183·3	169·4	148·6	138·8	160·00
Fresh Weight at Lifting ..	1417	833	527	436	803·8
(Branch Weight)/Total Weight	25·9	16·8	16·6	11·1	17·69
(Root Weight)/Total Weight	34·2	41·9	28·8	32·8	34·59
(F.W. at Planting)/(F.W. at Lifting)	8·59	4·98	3·54	3·21	5·078
$(\log_e W_1 - \log_e W_0) \times 100$ ..	194·2	158·2	124·0	115·0	147·85

the rootstocks increased more rapidly under an intermediate of No. XII than under one of No. IX ; as a result, the intermediate became much greater in cross section than the stock in trees of type B where the intermediate was No. IX and the rootstock No. XII ; whilst in combination C, where the intermediate and stock were Nos. XII and IX respectively, the rootstock became much greater than the intermediate. Furthermore, the No. IX intermediate in B became greater than the No. XII intermediate in C. The relative behaviour of intermediate and stock is brought out more clearly in Fig. 7, where the ratios of the mean values of the one to the other have been plotted. The value of the ratio is always lower for a No. XII stem piece than for a No. IX, whether both are on rootstocks of No. XII (A and B) or both on No. IX (C and D). The slope, or relative rate of change of cross sections, however, depends on the stock variety used as intermediate, the values remaining more or less unchanged when this was No. XII (A and C) but rapidly increasing when it was No. IX.

Weight of Tree and of Its Parts.

The mean values for the weights at planting in 1932 and at lifting in 1937 are given in Table VI. These refer to the trees from complete squares only. In each case four kinds of the trees can be placed in the same order of decreasing weight, A, B, C and D, but the differences between them were small at the time of planting and much larger at the time of lifting. This is brought out more clearly by examining the ratios of final to original weight ; the mean values of these are given in the fifth line of the Table. The A (XII/XII) trees had become about eight and a half times as heavy as they were when planted, the B (IX/XII) trees about five times their original weight, whilst the C (XII/IX) and D (IX/IX) had increased three and a half times respectively. For various reasons it is more usual and convenient to use the natural logarithms of these ratios as the measure of rate of growth. The value of this was determined for each of the sixty-four trees and their variance was analysed. The mean values are given in the last line of Table VI, and the significant differences are shown in the last column of Table V. The A (XII/XII) trees had the highest growth rate, the (IX/XII) trees had a higher rate than C (XII/IX) or D (IX/IX) trees and there was no significant difference between the rates for C and D.

Proportion of Branches and Roots.

The mean values for the branch weights and the root weights, each expressed as percentages of the total weights, are given in Table VI ; neither of them is of particular interest. The high value for the branches of A (XII/XII) is probably merely a reflection of the larger size of those trees, as

the ratio of branch weight to trunk weight tends to rise as the tree increases in size (4). The weight of the roots included that of the bases of the suckers, which in some cases was considerable ; differences between the mean values for the percentages will be due partly to differences in intensity of suckering.

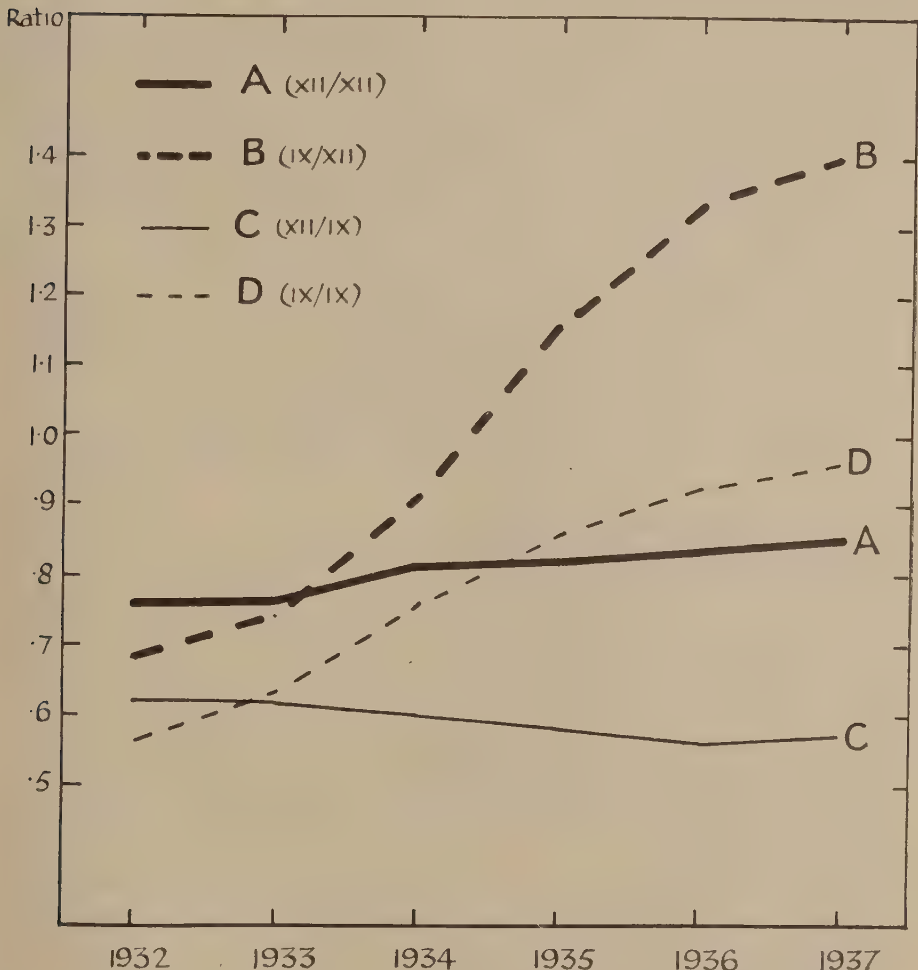

FIG. 7.

Ratio of Cross Section of Intermediate to that of Rootstock.

DISCUSSION.

So far as scion performance is concerned, the results described here are in full agreement with those already found by Knight (6, 7). The amount of wood growth and the number of fruit buds were largely influenced by the stock providing the intermediate stem piece and that providing the roots, but the latter tended to have the greater influence.

This close agreement is all the more striking because the scion variety and that of the vigorous stock were different, being Stirling Castle and No. XII instead of Lane's Prince Albert and No. I.

This new experiment was planned, moreover, to avoid two of the possible sources of ambiguity pointed out by Knight. The trees were double-worked in a single operation, and their number was fully adequate to give significant results, as the statistical analysis showed.

The other possible disturbing factors mentioned by Knight, were not, however, eliminated. The piece of stock stem, to which the roots were attached, was still present, and the graft unions were of a different character in the four types of tree ; for instance the lower unions were XII/XII, IX/XII, XII/IX and IX/IX respectively in types A, B, C and D.

The stem piece below the lower union was probably longer than in the earlier experiment ; this was arranged on purpose in order that annual measurements of its diameter might be possible.

The experiment was not designed to discriminate between the relative parts played by the stem piece and its roots in the rootstocks as normally constituted in this country ; this has already been partially explored (1, 3, 5, 8). It was designed mainly to examine the effect of an *additional* length of stock stem on scion performance and the possibility of combining the good points of two stocks by using one to provide the roots and the other to provide part of the stem.

Trees with two or more unions and composed of more than two varieties are being increasingly used in various parts of the world, as all forms of top-working and frame working amount in practice to the formation of double or multiple worked trees. It is desirable, therefore, to get as much information as possible on the effect of an intermediate piece of stem. The possibility that the graft unions, as such, may play an important part cannot be dismissed. Chang (2) has recently added to the evidence that these unions are important where incompatibility between scion and stock exists.

The peculiar swellings above the unions (see Fig. 2) when the lower component was No. IX, the difference in shape of these swellings when the upper component was No. XII or Stirling Castle, the absence of all swellings where both components were of the same stock variety, or one was Stirling Castle

and the other No. XII, all suggest that in unions of some kinds there are obstructions that are not present, or are not as pronounced, in others.

It is possible that the obstruction or resistance might be in the phloem in the first instance, for the swellings occurred above the unions and are not altogether unlike those that develop after the removal of a ring of bark. The swelling must, however, cause a marked distortion in the grain of the wood, and this might ultimately affect the rate of flow of fluids passing through the xylem. Dr. Warne, of Manchester University, is collaborating on this point by examining the conductivity of the trunks from these trees.

If the nature of the lower graft union plays an important part, this might be expected to show up as a significant difference in the " interaction " between rootstock and intermediate, though the converse does not necessarily hold good. For this difference is that between the thirty-two trees of types A and D, and those of B and C. Each group of the thirty-two consisted of sixteen with No. XII roots and sixteen with No. IX, also of sixteen with No. XII and sixteen with No. IX intermediates. The graft unions Stirling Castle/XII and Stirling Castle/IX were also sixteen in each group. The main difference was that in one group the stock and intermediate were both of the same variety, XII/XII and IX/IX, whilst in the other they were all different varieties, IX/XII and XII/IX, and the lower unions were therefore of a different nature.

Significant differences of a high value were found for interaction in summed wood growth and summed fruit buds (Table III) but not for fruit buds per metre wood.

These significant differences do not necessarily mean that the graft unions were responsible, for there are other ways in which rootstock and intermediate might interact.

Moreover it must be remembered that an unworked No. IX becomes a dwarf tree and produces fruit buds precociously, and there can be no question here of the effect of a graft-union.

The results are of sufficient interest to warrant further work on the same general line, and a new experiment has been planted in which the stock varieties XII and IX have been combined in all eight possible ways as rootstock, intermediate and scion.

ACKNOWLEDGMENTS.

The experiment has been in various hands since it was initiated. The details were planned by the present writer for Dr. Knight when he was away ill. The trees were made by the Propagation Section. Dr. Knight conducted the experiment on his return until his death at the beginning of 1935. The present writer took charge of it in 1935 and again in 1937, whilst Dr. Pearse

was responsible for it in 1936. The routine wood measurements and fruit bud counts were, for the most part, carried out by the recording staff of the Pomology Section. The photographs were taken by Miss K. Cornford.

SUMMARY.

1. The performance of four series of double-worked apple trees has been followed for six years.

2. The trees were double-grafted at a single operation and consisted of three portions: the scion (Stirling Castle), an intermediate piece of stock stem, and a root system belonging to a piece of stem (rootstock).

3. The stocks used were Malling No. XII (very vigorous) and No. IX (dwarfing), which were combined as root system and intermediate in all four possible ways, A (XII/XII), B (IX/XII), C (XII/IX) and D (IX/IX).

4. The trees on No. XII root systems produced significantly more wood growth and significantly fewer total fruit buds and fruit buds per metre wood than those on No. IX root systems.

5. The trees with No. XII intermediates produced significantly more wood growth and significantly fewer fruit buds per metre wood than those on No. IX intermediates. Differences in total fruit buds were not significant.

6. There was significantly more wood growth and there were fewer total fruit buds where stock and intermediate were of the same variety than when they were different. This significant effect of interaction may have been due to the nature of the graft unions, though not necessarily so. The difference in fruit bud per metre wood was not significant.

7. Pronounced swellings occurred above graft unions where the lower component was No. IX and the upper Stirling Castle or No. XII. The swellings were morphologically different in these two cases. No swellings occurred above unions between like components or between Stirling Castle and No. XII.

8. The cross section of the intermediate was determined by the rootstock below it, and that of the rootstock by the intermediate above it. As a result the No. IX intermediate in trees of type B became larger than the stems of the No. XII stocks below.

REFERENCES.

(1) *Beakbane, A. B., Hatton, R. G.* and *Amos, J.* Mechanism of Rootstock Effect. East Malling Res. Sta. Ann. Rpt. for 1936 (1937), 31.

(2) *Chang, W. T.* Studies in Incompatibility between Stock and Scion, with Special Reference to Certain Deciduous Fruit Trees. Journ. Pom. & Hort. Sci., 1938, **15**, 257.

Scion. *Intermediate.* *Rootstock.*
A. Stirling Castle. No. XII. No. XII.

Scion. *Intermediate.* *Rootstock.*
B. Stirling Castle. No. IX. No. XII.

Scion. *Intermediate.* *Rootstock.*
C. Stirling Castle. No. XII. No. IX.

Scion. *Intermediate.* *Rootstock.*
D. Stirling Castle. No. IX. No. IX.

FIG. 1.

Typical specimens of the four kinds of tree. Each consisted of three parts, *Scion* (Stirling Castle), *intermediate stem piece* (No. XII or No. IX) and *rootstock* (No. XII or No. IX). The two graft unions were made at the same time. All figures are on the same scale.

Scion. *Intermediate.* *Rootstock.*
A. Stirling Castle. No. XII. No. XII.

Scion. *Intermediate.* *Rootstock.*
B. Stirling Castle. No. IX. No. XII.

Scion. *Intermediate.* *Rootstock.*
C. Stirling Castle. No. XII. No. IX.

Scion. *Intermediate.* *Rootstock.*
D. Stirling Castle. No. IX. No. IX.

FIG. 2.

Trunks of three typical specimens of each kind of tree, all on approximately the same scale. White marks are where diameters were measured on scion, intermediate and rootstock. Upper (scion/intermediate) graft union is between top and middle marks; lower (intermediate/rootstock) graft union is between middle and bottom marks.

(3) *Hatton, R. G.* The Influence of Vegetatively Raised Rootstocks upon the Apple, with Special Reference to the parts played by the Stem and Root Portions in affecting the Scion. Journ. Pom. & Hort. Sci., 1931, **9**, 265.

(4) ———. Apple Rootstock Studies. Effect of Layered Roots upon the Vigour and Cropping of Certain Scions. Journ. Pom. & Hort. Sci., 1935, **13**, 293.

(5) *Hatton, R. G.* and *Amos, J.* Analysis of Rootstock Influence including the Reciprocal Effect of Scion on Root. East Malling Res. Sta. Ann. Rpt. for 1934 (1935), 34.

(6) *Knight, R. C.* Preliminary Observations on the Causes of Root Influence in Apples. East Malling Res. Sta. Ann. Rpt. for 1925 (II Supplement) (1927), 51.

(7) ———. Further Observations on the parts played by Root and Stem in Stock Influence. East Malling Res. Sta. Ann. Rpt. for 1933 (1934), 114.

(8) *Vyvyan, M. C.* The Effect of Scion on Root—III. Comparison of Stem and Root Worked Trees. Journ. Pom. & Hort. Sci., 1930, **8**, 259.

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SPOTTING AND OTHER EFFECTS ON APPLES IN STORAGE DUE TO VOLATILE PRODUCTS FROM RIPE APPLES OF OTHER VARIETIES STORED WITH THEM

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and

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In the course of a systematic survey of the effects of the three variables, carbon dioxide, oxygen and temperature, on the storage life of apples, the present writers observed certain unexpected results following the storage of ripe, post-climacteric Worcester Pearmain apples with later maturing varieties such as Bramley's Seedling, King Edward VII, Laxton's Superb and Cox's Orange Pippin. These results were recorded in the Annual Report of the Food Investigation Board for 1934. The following quotations are taken from page 104 of this report:—

"In the season under review, one of the varieties, Worcester Pearmain, was gathered at the peak of respiratory activity, i.e. the apples were post-climacteric and had a strong smell, while their ground colour was beginning to turn yellow. From what is now known these apples were undoubtedly evolving ethylene in sufficient quantities to stimulate the climacteric in all the other varieties stored with them, and this could have happened during the first few hours after the storage-cabinets had been closed, and before the fruit had cooled or the artificial atmospheres become established."

"The ripe Worcester Pearmains had a harmful effect on the other varieties in the same cabinet, not only in accelerating their ripening through the effect of ethylene upon the climacteric, but also in causing severe spotting round the lenticels, which, although definitely functional in origin, was often followed by fungal invasion of the injured tissues. This spotting was particularly bad in the Bramley's Seedlings and the Cox's Orange Pippins."

In order to confirm and extend the information on this important subject, further experiments were carried out during the past season. For these experiments Bramley's Seedling, King Edward VII and Laxton's Superb were used

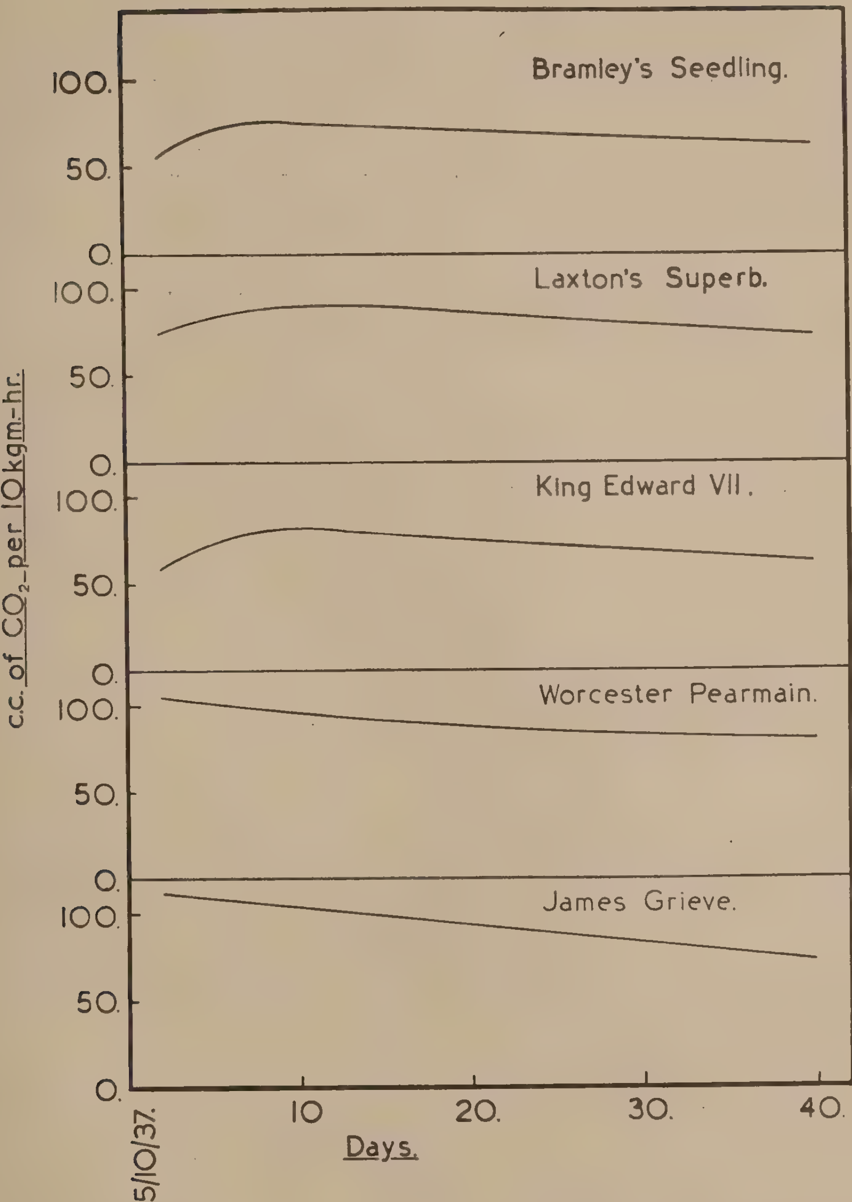


FIG. 1.

Respiratory activity at 12° C. of the three varieties of apples (Bramley's Seedling, Laxton's Superb and King Edward VII), stored in the pre-climacteric condition and of the two varieties (Worcester Pearmain and James Grieve) stored in the post-climacteric condition, from the time when they were placed in store.

as the test varieties and were placed in store on October 4th, 1937, in the pre-climacteric condition (Text Fig. 1). The varieties used as toxic agents were Worcester Pearmain and James Grieve. When placed in store both of these varieties were yellow, ripe and greasy and had a strong aroma. Records of their respiratory activity showed that they had passed the peak of the climacteric (Text Fig. 1). While the Worcesters had been allowed to remain on the trees until they were required, the James Grieves had been stored in air at 50° F. (10° C.) since the date of gathering (9/9/37).

The storage temperature was 39° F. (4° C.). Parallel series of experiments were carried out with fruit stored in air and in gas (10 per cent. carbon dioxide + 10 per cent. oxygen + 80 per cent. nitrogen). Each sample unit consisted of four trays of twenty apples. The fruit was stored in gas-tight metal cabinets and the storage atmospheres, both air and gas, were made up by mixing the pure gases in the correct proportions. These gas-mixtures were passed through the cabinets at a steady rate, just sufficient to prevent a rise in the concentration of carbon dioxide (due to the respiration of the fruit) of more than a fraction of a per cent. between entry and exit. Before entering the cabinets the atmospheres were humidified to approximately 92 per cent. saturation.

In both series, i.e. air and gas, there were three cabinets, the first containing trays of test fruit alone; the second, trays of test fruit alternating with trays of Worcester Pearmain; and the third, trays of test fruit alternating with trays of James Grieve. In every case a comparison was made between unwrapped fruit and fruit wrapped in oiled paper.

It is necessary to point out here that in these experiments, in which the atmospheres in the cabinets were obtained and maintained by passing gas mixtures of the required composition through them at a given rate, the gas-storage conditions are not exactly the same as those obtained in commercial practice by the method of restricted and controlled ventilation. In the former the apple vapours are constantly being swept away, and conditions as regards these vapours are produced approximating to those obtaining in air-storage. In commercial gas-stores, on the other hand, the apple vapours tend to accumulate and may reach relatively high concentrations.

It was also desired to determine whether the effect of treating the test fruit with ethylene would be similar to that of storing together pre-climacteric and post-climacteric apples. This test was included only in the series with fruit stored under gas-storage conditions. Ethylene, at the rate of 1 part in 500, was added to the storage atmosphere for three weeks (November 15th to December 5th).

All the fruit was removed from storage and examined on the same date (31/1/38), with the following results.

LENTICEL SPOTTING.

Bramley's Seedling. With this variety the broad result was striking. The Bramley's in the cabinets that contained Worcester Pearmain or James Grieve apples, and also those that had been treated with ethylene, showed severe lenticel spotting (Plate I, Fig. 1). The control Bramley's in air and in gas were practically free from this type of injury. The spotting brought about by the presence of the ripe apples was definitely less pronounced on the wrapped fruit than on the unwrapped fruit. On the other hand, the spotting associated with the ethylene treatment, whilst identical in appearance with that caused by the ripe fruit, was not affected by the oiled wrappers. The effect of the Worcesters in causing spotting was much more pronounced than that of the James Grieve variety.

The marked difference between the controls, both in air- and in gas-storage, and the corresponding samples of fruit that had been exposed to the vapour of ripe post-climacteric Worcesters is clearly brought out in Plates II, III and IV, Figs. 3, 4 and 5. Plate III, Fig. 4, shows the condition of the fruit after a further period of storage in air at ordinary room temperature. At this temperature invasion of the injured tissues by fungi takes place very rapidly.

The effect of the ethylene treatment as regards lenticel spotting is illustrated in Plate V, Fig. 6.

Laxton's Superb. Essentially similar results were obtained with this variety, the main difference being that the James Grieve and Worcester Pearmain apples were equally active here in producing the injurious effect. The type of spotting that developed on the Laxton's is shown in Plate I, Fig. 2.

King Edward VII. Neither the vapour from the ripe fruit nor the ethylene treatment had any effect, as regards spotting, on the King Edward VII apples. No spotting developed on this variety under any of the storage conditions tested.

Worcester Pearmain. It is interesting to note that these apples showed little or no signs of spotting, although they were, of course, over-ripe and "wasty" at the time the experiment was terminated.

James Grieve. The condition of the James Grieve apples at the end of the experiment was very similar to that of the Worcesters. On this variety, also, little or no spotting had developed.

SCALD.

A point of some interest emerging from these experiments was that the Bramley's developed a small amount of superficial scald under the gas- but not under the air-storage conditions. The scald amounted only to about 2 per cent. of the surface of the fruit, but, contrary to expectation, there was

no indication that wrapping in oiled paper had any effect in controlling it. From previous experience it can be assumed that if the gas-storage atmosphere employed had been obtained by the method of restricted and controlled ventilation, as practised in commercial gas-storage for fruit, and not by the continuous flow method, severe scald would have developed on the unwrapped apples, whilst the wrapped fruit would have shown freedom from this disease. Such a result was, in fact, obtained in the course of the experiments of 1933-34 referred to above (1). It must be concluded, therefore, that in the present experiment the continuous through ventilation, which was the same in both air- and gas-storage, was sufficient to prevent any significant difference between the wrapped and the unwrapped fruit in the concentration at the surface of the fruit of the volatile substances responsible for scald. It has been shown elsewhere (2) that, other conditions being equal, scald is more severe the higher the concentration of carbon dioxide in the storage atmosphere.

A further point of interest is that the presence of the ripe, post-climacteric apples (Worcester Pearmain or James Grieve), which were actively producing the volatile substances responsible for the characteristic aroma of these varieties at the time the fruit was placed in storage, had no effect on the development of scald; nor had the ethylene treatment. It is a well-known fact that superficial scald is usually associated with immature fruits under conditions of restricted ventilation. The present evidence suggests that the volatile substances responsible for scald are produced only during the pre-climacteric phase and possibly also during the climacteric rise in respiratory activity.

Effects on Rate of Ripening.

On the basis of results previously obtained (1) it was expected that the presence of ripe Worcester Pearmain or James Grieve apples would accelerate the rate of ripening of the test fruit. In the present experiment, however, the presence of the ripe apples appeared to have little effect on the rate of ripening of the test fruit, as judged by ground-colour, hardness, taste, etc., either in air- or in gas-storage.

It was also expected that ethylene would accelerate ripening, since it has been shown that this gas stimulates the onset of the climacteric rise in respiratory activity in air. More recent experiments designed to determine the effect on respiratory activity of ethylene in the presence of carbon dioxide, the results of which are reported elsewhere (3, p. 113), have shown that stimulation by ethylene without recovery can occur even under gas-storage conditions. The present experiments have yielded a clear cut result in demonstrating the stimulating effect of ethylene on the ripening of apples under gas-storage conditions. The apples were softer and yellower at the time of the examination than any of those in the gas-stored series that had not been treated with ethylene.

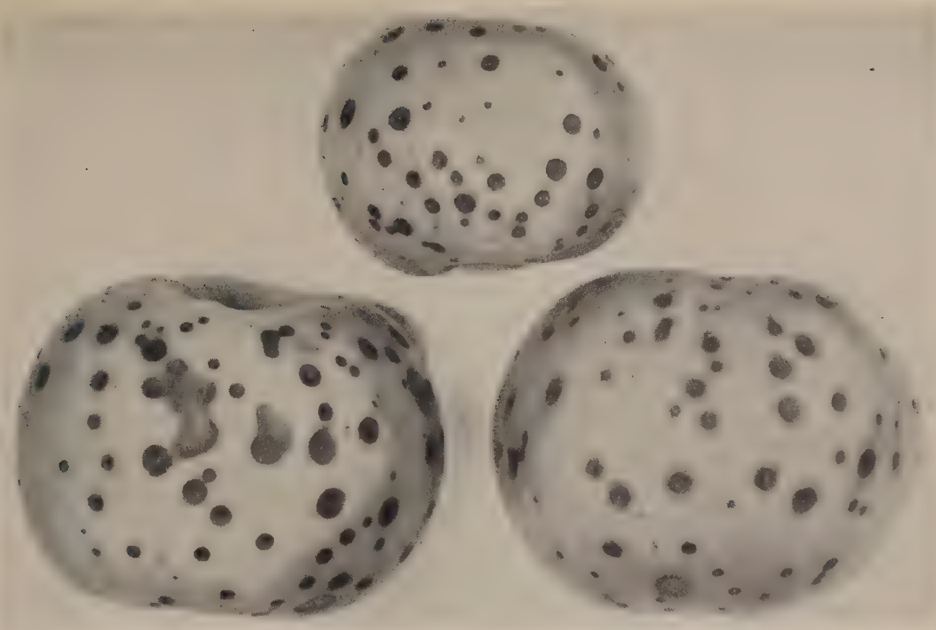
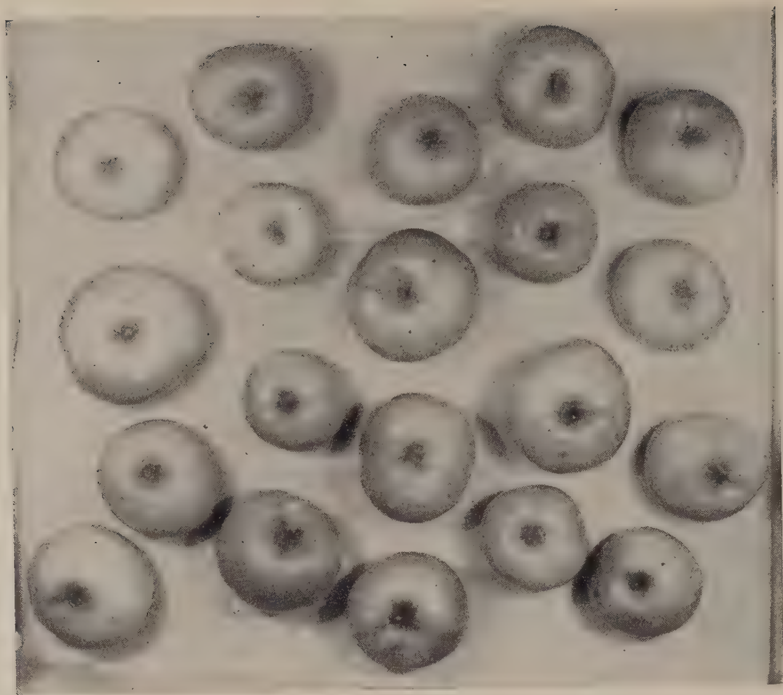
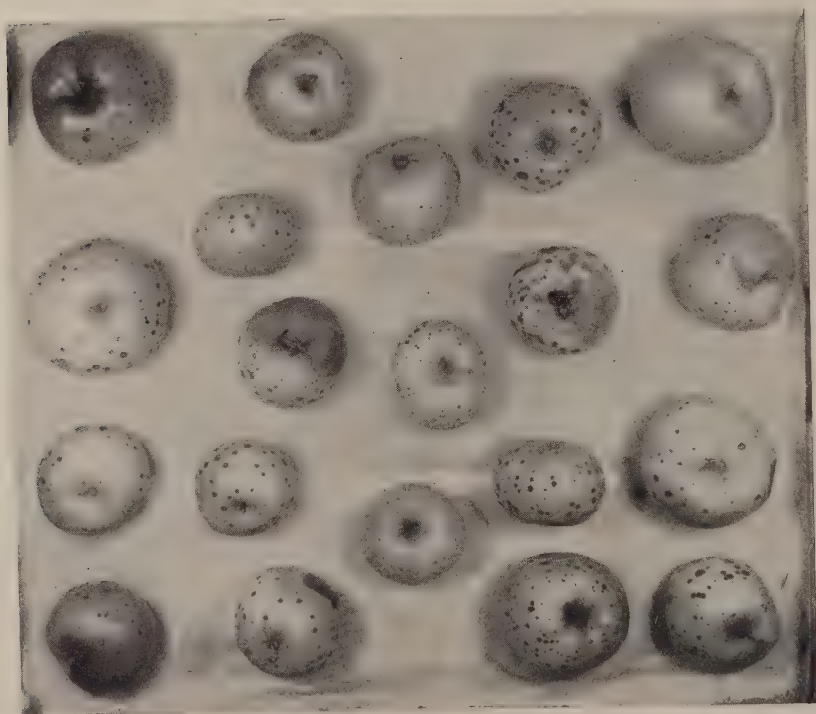


FIG. 1. Lenticel spotting on Bramley's Seedling apples which had been stored in air at 40° F. with ripe (i.e. post-climacteric) Worcester Pearmain apples.

FIG. 2. Lenticel spotting on Laxton's Superb apples which had been stored in air at 40° F. with ripe (i.e. post-climacteric) James Grieve apples.

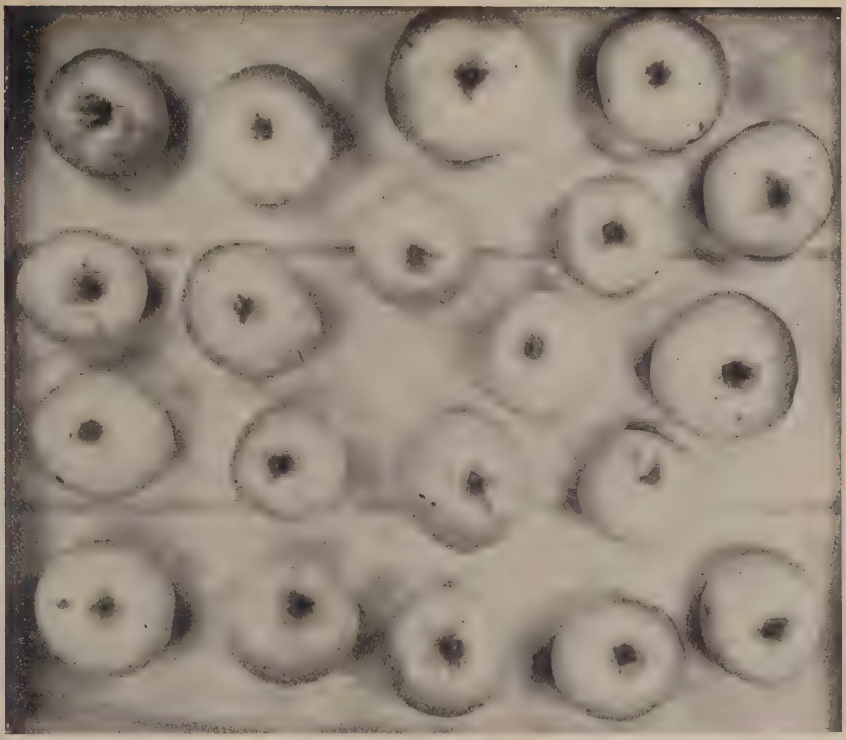


A.



B.

FIG. 3. Condition of Bramley's Seedling apples after 17 weeks' storage in air at 40° F. A. Stored *without* Worcester Pearmain. B. Stored *with* Worcester Pearmain.



A.

B.

Fig. 4. A. Condition of the Bramley's Seedling apples in Fig. 3A, after a further 2 to 3 weeks' storage at ordinary room temperature.
B. Condition of the Bramley's Seedling apples in Fig. 3B, after a further 2 to 3 weeks' storage at ordinary room temperature. Note development of rotting by fungi.

A.

B.

FIG. 5. Condition of Bramley's Seedling apples after 17 weeks' gas-storage at 40° F.
A. Stored *without* Worcester Pearmain. B. Stored *with* Worcester Pearmain.

PLATE V.

A.

B.

FIG. 6. Condition of Bramley's Seedling apples after 17 weeks' gas-storage at 40° F. A. *Not treated* with ethylene.
B. *Treated* with ethylene (1 in 500) for 3 weeks (November 15th to December 5th, 1937).

DISCUSSION.

The fact that ethylene and the vapours from ripe apples produced the same effect on stored fruit as regards spotting, but different effects as regards ripening, should have some significance. As the result of the recent work of Gane (4) it can be assumed with confidence that the vapours of ripe apples contain ethylene as one of their components. It is tempting, therefore, to conclude that the spotting was due in both cases to the presence of this gas.

The absence of a stimulating effect on ripening of the volatile products from the ripe apples may be attributed to a difference in the duration of exposure or in the concentration of ethylene present as compared with the experiment in which ethylene was actually added to the storage atmosphere.* An alternative possibility is that the ripe apples produced a volatile substance having a retarding effect on ripening. Supporting the latter explanation is the fact that the apples gas-stored with the ripe fruit showed no signs of being riper than the control apples ; in fact, as far as flavour and taste were concerned, they appeared to be less ripe than the controls in gas-storage.

SUMMARY.

Skin spotting lesions, originating at the lenticels, have been produced on apples as a result of storing them with ripe apples of another variety. A similar type of spotting was induced by storing apples in an atmosphere containing ethylene (1 part in 500). Varieties differ markedly in their susceptibility to this type of spotting injury.

LITERATURE CITED.

(1) *Kidd, F.* and *West, C.* Gas-Storage of Apples. Ann. Rpt. of the Food Invest. Board, London, for the Year 1934, p. 103.

(2) ———. Gas-Storage of Fruit. III. Lane's Prince Albert Apples. Journ. Pom. & Hort. Sci., 1933, **11**, 149.

(3) ———. The Effect of Ethylene on the Respiratory Activity and the Climacteric of Apples. Ann. Rpt. of the Food Invest. Board, London, for the Year 1937, p. 108.

(4) *Gane, R.* The Formation of Ethylene by Plant Tissues, and its Significance in the Ripening of Fruits. Journ. Pom. & Hort. Sci., 1935, **13**, 351.

(5) *Molisch, H.* Der Einfluss einer Pflanze auf die andere, Allelopathie. Jena, 1937.

* In a recent publication, Molisch (5) presents data which show that the vapours from ripe apples behave as regards their effect on various growth phenomena, e.g. the growth of pea and vetch seedlings, in the same way as certain organic poisons. In small doses they act as stimulants, whereas in larger doses they have an inhibiting effect. Still larger doses are definitely injurious.

(*Received* 24/6/38.)

BACTERIAL DISEASES OF STONE-FRUIT TREES IN BRITAIN

VII. THE ORGANISMS CAUSING BACTERIAL DISEASES IN SWEET CHERRIES

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INTRODUCTION.

THE bacterial diseases of the sweet cherry in this country have been under observation since 1919, when dead and dying branches of Black Tartarian cherry trees at Ham Street, near Ashford, Kent, were found to have lesions containing dense masses of bacteria. These diseases are now known to be widespread and responsible for great losses, particularly in plantations of young cherry trees.

The work of various investigators on the Continent and in America in relation to bacteriosis in cherry trees has already been reviewed (8), and here it is necessary only to point out that the first clear evidence of bacteria causing disease in cherry trees was published in 1906 by two German workers, Aderhold and Ruhland (1). In 1911 bacterial gummosis of cherries was described as occurring in Oregon (3) ; since then it has been found in several parts of North America and has been studied by various workers, particularly E. E. Wilson (5).

Two recent papers by Grubb (4) and the present writer (8) described field observations on bacteriosis in sweet cherries in Britain, with detailed accounts of the symptoms, and a discussion of the relative susceptibility of varieties. It was stated that bacterial infection in cherries results in (1) stem and branch cankers, (2) the death of buds and spurs, (3) shoot wilt, (4) leaf spotting and (5) fruit spots, and that these diseases in this country are caused by two species of bacteria, *Pseudomonas prunicola* and *P. mors-prunorum* ; but experimental evidence that these organisms are the actual cause of these diseases in cherry trees was not presented.

The two species have already been fully described (6, 7) in relation to diseases which they cause in plum trees. In continuation of that work organisms have been isolated from cherry trees and have been compared with those previously obtained from plum trees with regard to their behaviour when grown in certain culture media, and inoculation experiments have shown that these organisms are able to infect cherry trees and produce the diseases described.

ISOLATION AND CULTURAL STUDIES.

In all, about eighty isolations have been made from lesions on various parts of sweet cherry trees, i.e. cankers on stems, branches and twigs, lesions

on young shoots, dead buds, leaf and fruit spots. The isolations were made on plates of nutrient agar containing 5 per cent. saccharose, as this was found preferable to plain nutrient agar for distinguishing the parasitic organisms from chance saprophytes. As mentioned in a previous paper (7), the plum organisms on this medium produce raised, whitish, circular colonies, with close radiating lines as seen when the young colonies (about two days old) are examined under a microscope of fairly low power ($\frac{2}{3}''$ objective), from the lower side of plate cultures ; and this character was used for diagnosis when selecting for study the strains from cherry.

Various cultural tests have been applied to the cherry organisms and it has been found that the strains isolated can readily be relegated into two groups, with characters corresponding to those of *Pseudomonas prunicola* and *P. mors-prunorum*, respectively. It has been pointed out (7) that there are certain tests by which these two organisms can be distinguished from each other, and in the later isolations these tests alone were applied for rapid identification.

Usually no great trouble is experienced in obtaining pure or nearly pure cultures on plates at a first attempt, if suitable material is selected. Leaf spots and lesions on young shoots present no difficulty. Lesions on twigs, branches and stems are suitable in May and June ; they are then fairly easily recognized and the bacteria in them are still alive. During the summer the organisms within the tissues of the cankers tend to die out, and attempts at isolation in late summer are usually unsuccessful, though isolations have been obtained in early August.

In preparing the isolations from leaf spots all that is necessary is to cut out, with sterile scissors, one of the spots, place it in a little sterile water in a watch-glass and tear the infection spot across with needles. The bacteria gradually ooze out and after an hour or so plates may be prepared in the usual way from the bacterial suspension. It is generally unnecessary to sterilize the surface of a leaf before cutting out a spot. When the excised spot is immersed in the water and cut across, it is advisable to examine the cut edges under a low power of the microscope to see whether bacterial slime is oozing out into the water ; if none can be observed another spot should be selected.

With stem and branch cankers the borders of the lesion are first cleansed by wiping with a wad of cotton wool moistened with alcohol. The surface layers of the bark are then cut away with a flamed scapel and a small piece of the infected tissues is transferred to sterile water in a watch-glass. This again may be examined under a low power to see if bacterial masses are present.

The plates are incubated at 25° C., and when the colonies are about forty-eight hours old they are examined for the radiating structure mentioned. Transfers are made on the third day to nutrient broth containing 5 per cent.

saccharose. The plates should not be kept longer than this before transfers are made since *Pseudomonas mors-prunorum* soon loses its vitality on such plates and is usually dead by the sixth day ; even on the fourth day transfers from colonies on rather thickly sown plates sometimes give no growth.

When the tubes of nutrient broth with saccharose are a few days old they are examined by reflected light using a rather dark background ; the cultures of *P. prunicola* have then a slight yellowish tinge and are more or less translucent, while those of *P. mors-prunorum* are white and somewhat opalescent.

Slopes of nutrient agar with 5 per cent. saccharose are then inoculated and incubated at 25° C. for six days, when transfers are made from the middle of the slopes to nutrient broth. With *P. prunicola*, the transfers readily result in growth, which becomes evident within one or two days, but with *P. mors-prunorum* there will be no growth, showing that the cultures are already dead.

Another test which has been applied to most of the strains isolated is to inoculate a slope of nutrient agar containing 2 per cent. lactose and bromo-cresol-purple as indicator. All strains cause this medium to show an alkaline reaction within two days and this persists in the cultures of *P. prunicola*. With strains of *P. mors-prunorum*, however, the reaction becomes reversed ; the yellowish coloration appears at the top of the slope and gradually extends downwards throughout the medium to the base. This reversal may start within four days, but with some strains a fortnight or so elapses before any change is detected. With strains of *P. prunicola* there is no reversal even in cultures kept for two months or more. This medium as usually prepared contains only 1 per cent. of lactose, but as shown in describing strains of *P. mors-prunorum* from plum, the results are irregular with the lower concentration of lactose. The same inconsistency was found in the cherry strains of that organism. In the earlier tests, using 1 per cent. lactose, some strains, which from other characters would be referred to *P. mors-prunorum*, did not produce the reversal of reaction, but later it was found that those strains, too, produced acidity in the medium when it contained 2 per cent. lactose.

Other differences which were used for confirmation of diagnosis were obtained by growing the various strains in Uschinsky's solution and in beef extract (prepared from fresh meat). In these media a distinct yellowish coloration appears with *P. prunicola*, but not with *P. mors-prunorum*.

INOCULATION EXPERIMENTS.

(1) On Cherry.

Many inoculations with the organisms isolated have been carried out on cherry trees—on leaves, shoots, branches and stems. Not all the experiments yielded positive results but definite lesions were obtained from inoculations, in controlled experiments, with about thirty of the strains isolated.

The experiments giving the most striking results are described below. They have been carried out at various times from 1925 to 1932. The number given to an experiment indicates the year in which it was carried out and the serial number of the experiment that year. This is followed by the name of the organism used (as deduced from the cultural tests mentioned above), and the kind of lesion from which it was isolated.

Expt. 25/5. Strain VIIa, *P. prunicola*, from a cherry leaf spot.

Six shoots were inoculated, each at a single puncture on May 19th, and six control shoots were also punctured but not inoculated.

Within three weeks lesions from 6 mm. to 6 cm. in length developed on all the inoculated shoots ; those shoots with the longest lesions were distorted and growth was arrested. No lesions developed on the control shoots.

Expt. 26/4. Strain as in Expt. 25/5.

Buds on a cherry twig were inoculated on March 12th by inserting a drop of a suspension of the organism between the opening leaves ; some of the buds were punctured, others left uninjured.

As the inoculated buds grew out most of their leaves showed bacterial spotting or shot holes. Other buds on the same twigs also bore infected leaves, but there was no infection on control twigs.

Expt. 26/23. Strain XX, *P. mors-prunorum* from the stem of a young cherry tree.

In October six young cherry trees were each inoculated at one place on the stem, six others at two places (eighteen inoculated wounds), while of ten control trees, five were wounded (but not inoculated) at one place, and five at two places each on the stem to correspond to the inoculated trees (fifteen control wounds).

By the following summer all the inoculated wounds had produced cankers ; these were very variable in length, the shortest measuring 5 cm., and the longest over 30 cm., most of them being from 10 to 25 cm. long.

In the control trees one became infected and died, and on another a small sunken area appeared, but on the rest no cankers appeared, and thirteen out of the fifteen control wounds became normally covered with callus with no trace of cankering. As each control tree had an inoculated tree next to it in the row, it is not surprising that there was some infection in the control trees, but the fact that thirteen out of fifteen control wounds healed normally while all the inoculated wounds produced cankers is clear proof of the parasitism of the organism used in the experiment.

Expt. 26/30. Strain XIIa, *P. prunicola* from a cherry leaf spot.

Cherry buds were inoculated in November by putting a little bacterial slime on the buds and making slight punctures through the slime into the buds. Other buds were inoculated with the slime without puncturing. For controls

some buds were punctured, others left unpunctured. The buds on one tree only were used.

The buds were examined in the following April when it was found that nine out of twenty-two inoculated and punctured were dead, two were retarded in growth, while the rest grew out normally without any definite leaf spotting. The buds inoculated without puncturing and all the control buds also grew out normally.

Expt. 27/6. Strains XX and XXR1, *P. mors-prunorum*, the former from a natural infection on a cherry stem, the latter from a tree inoculated with XX in Expt. 26/23.

Buds on four one-year-old cherry twigs (variety Napoleon) were inoculated in April. On each twig eight buds were inoculated with drops of a suspension (in water) of the organism, alternate buds being punctured. Two twigs were inoculated with Str. XX and two with XXR1.

By June the buds had grown out to produce shoots; of those shoots from buds inoculated and punctured, all, with one exception, bore brown and withered or spotted leaves; those inoculated without puncturing mostly grew out normally, only a few of the leaves showing spotting. Later (August), seven of the shoots from punctured inoculated buds were completely withered. The buds of control twigs, on which alternate buds were punctured without inoculation, produced normal shoots with healthy leaves.

Expt. 27/22. Strain XXR2, *P. mors-prunorum*. This was a re-isolation of strain XX from a leaf spot obtained from Expt. 27/6.

In November three branches were each inoculated at two places. Each inoculation was effected by making a ∧-shaped cut, turning back the tongue of bark, and inserting a drop of a suspension of the organism. Control branches were similarly cut but not inoculated.

By the following summer cankers 10-15 cm. long had developed on the inoculated branches, and two of them were girdled and killed. Small canker-like areas 2·5-6 cm. long appeared on the control branches, but these became covered with callus.

Expt. 28/17. Strain XXIII, *P. mors-prunorum* from a cherry leaf spot.

Two current year's shoots were inoculated in June at two places each by punctures. Two other shoots, as controls, were punctured without inoculation.

Elongated black lesions 0·5-1 cm. long developed on the inoculated shoots while the control spots healed normally without any discoloration.

Expt. 29/4. Strain XXVII, *P. prunicola* isolated from a cherry branch.

Leaves were inoculated in May by spraying two leafy twigs of a cherry tree in the greenhouse with a suspension of the organism in water, and two other twigs were sprayed with sterile water as controls.

In twelve days some of the leaves on the inoculated twigs showed typical spotting, while the foliage on control twigs was quite healthy.

Expt. 29/6. The same strain was used as in Expt. 29/4 for spraying leafy twigs in the open.

The results were more striking than in the greenhouse, for many of the leaves on the inoculated twigs showed blackened spots and blotches, while on three of the twigs the terminal shoot was blackened and withered. The foliage on the control branches showed little or no spotting. From one of the leaf spots the organism was isolated as strain XXVIIR (see Expt. 29/28).

Expt. 29/26. Four strains were used:

XXIV. *P. mors-prunorum* from a cherry leaf spot.
XXV. ,, ,, ,, ,, ,, ,, ,,
XXIX. ,, ,, ,, ,, ,, stem.
XXX. *P. prunicola* ,, ,, ,, ,,

In November inoculations were made on a number of cherry branches all on the same tree, one branch being used for each strain, with two inoculations on the two-year-old wood and one on a current year's twig. One branch was used as control.

In the following summer the control wounds showed only a trace of gum and no cankers had developed, while at the inoculated wounds there was copious gum and three of the strains had produced definite cankers from 1-6·5 cm. in length; the other strain (XXIV) produced no definite cankers.

Expt. 29/27. Four strains used:

XXVIII. *P. mors-prunorum* from a cherry spur.
XXXIII. ,, ,, ,, ,, ,, leaf spot.
XXXVI. *P. prunicola* ,, ,, ,, stem.
XXXVIII. *P. mors-prunorum* ,, ,, ,, ,,

In November inoculations were carried out as in Expt. 29/26 but on another tree.

Again there was little gumming at the control wounds, but one small canker had arisen. On the inoculated branches there was copious gum with well marked cankers and three of the branches were killed.

Expt. 29/28. Two strains used:

XXVIIR. *P. prunicola*, a re-isolation of XXVII from a leaf spot in Expt. 29/6.
XXXII. *P. mors-prunorum* from a leaf spot.

In November, branches of Napoleon cherry trees were inoculated, eight with strain XXVIIR, eight with strain XXXII, while eleven were cut but not inoculated to serve as controls.

When examined in July 1930, only two of the control wounds had a little gum, the rest being quite dry. All the inoculated branches showed copious gum

and cankers 5-15 cm. long had developed. Eleven of these branches were quite dead while four others were also girdled and bore curled leaves.

Expt. 29/30. Strains as in 29/28.

Inoculations were made on the stems of young Napoleon cherry trees in November. Eight trees were controls, eight were inoculated with strain XXVIIR and eight with XXXII.

In the following June one control tree was dead, but the other controls showed no cankers and the wounds were being covered with callus. All the inoculated trees bore cankers 7-25 cm. long and three were dead.

As in Expt. 26/23, one control tree became cankered and died ; this must have been a chance infection, for none of the other control trees showed any trace of infection while all the inoculated trees produced well-developed cankers, starting at the points of inoculation.

Expt. 32/2. Five strains of *P. mors-prunorum*:

XLII	isolated	from	a	cherry	shoot.
XLIII	,,	,,	,,	,,	stem.
XLIV	,,	,,	,,	,,	twig.
XLVII	,,	,,	,,	,,	,,
XLVIII	,,	,,	,,	,,	leaf spot.

Inoculations were made in October on two-year-old branches and one-year-old twigs.

The trees were examined in August 1933. There were no cankers on the control branches ; some of the wounds had produced gum, but most of them had healed without exudation of gum. On the inoculated branches there was great variation according to the strain used. Strain XLII produced no infection, and strain XLIII produced only one small canker. Strains XLIV and XLVII yielded four and two cankers respectively (from six inoculations with each). From eight inoculations with XLVIII eight cankers resulted, four of them girdling and killing the branches. The cankers did not increase further and those which had not girdled the branches developed callus.

Expt. 32/6. Two strains of *P. prunicola*:

XLV	isolated	from	a	stem	canker.
XLVI	,,	,,	,,	node	lesion.

Inoculations were made in October on cherry branches, six with each strain, and there were six controls.

By the following August no cankers and no gum occurred on the control trees. The inoculations with strain XLV had produced six cankers, 2·5-6 cm. long with copious gum. Those with strain XLVI showed five cankers 6-15 cm. long, four of them girdling the branches.

As in Expt. 32/2, the cankers made no further progress and callus developed around their edges, so tending to heal the lesions.

(2) CROSS-INOCULATIONS.

Inoculations have been carried out with both *Pseudomonas prunicola* and *P. mors-prunorum*, using plum strains for inoculations on cherry, and cherry strains on plum. Positive results have been obtained showing that the organisms from each host can infect the other. Generally, however, these cross-inoculations have not been so successful as inoculations with strains isolated from the same host species. This suggests some degree of biological specialization among the strains, but the evidence is not conclusive, since in most of the experiments no direct comparison was attempted. In one experiment, however, direct comparison was possible, since strains of *P. mors-prunorum* from the different hosts were used on separate branches of one and the same tree, four inoculations being made with each strain.

With the plum strain, inoculations on plum produced cankers 4-10 cm. long, two branches being killed; the same strain on cherry produced cankers only 1-4 cm. long. The cherry strain on cherry produced cankers killing all four branches, but with this strain on plum two inoculations produced no cankers and the two others small cankers 1·5 and 2 cm. long.

It will be seen that in this experiment the plum strain was more virulent on plum than on the cherry strain, while the cherry strain was more virulent than the plum strain when inoculated into cherry.

DISCUSSION OF INOCULATION RESULTS.

Strains isolated from lesions on various organs were able to infect other organs, so it can be assumed that the same organism is able to attack stems, branches, shoots and leaves. This is true for both *Pseudomonas prunicola* and *P. mors-prunorum*. The most important relation is that existing between the cankers on stems or branches, and the spots on the leaves, since these are two distinct phases of the disease. The organism passes the winter and spring in the stems and branches, producing lesions which appear as cankers during the summer. The cankers are most evident at those lesions which only partly girdle a branch or stem. They arise by the continued growth of the tissues around the margins of the lesions the centres of which are dead, and the bark is thus caused to split. The bacteria within these cankers die out in summer, but in the meantime the leaves become infected and carry the organism over in leaf spots until the autumn, when stems and branches again become susceptible.

It is not known how infection of the leaves in spring arises. There are two possibilities: (1) that the organism is carried from the stem and branch lesions to the leaves by insects or is splashed on to the leaves by rain; (2) that the organism passes the winter in, or on, the buds and infects the leaves as they expand. Evidence that the organism is transported by insects is not yet forthcoming, but it would seem possible that aphides, ants and other crawling

insects would catch up the bacteria on their bodies and legs and so carry them from stem and branch lesions to leaves, and from leaf spots to other leaves. That leaves may become infected by rain splashing the organism from cankers to leaves seem certain. Leaf spotting has at times been seen to be most severe in the vicinity of branch lesions, and particularly on leaves immediately below the lesions. Moreover, in certain inoculation experiments (29/4, 29/6) infection of the leaves arose after they had been sprayed with water containing a suspension of a culture of the organism. There is no visible exudation of bacteria on the leaves, but if infected leaves are removed in wet weather and the spots teased out in water under the microscope, bacteria may often be seen moving rapidly about in the water. Hence it is highly probable that the organisms come to the surface and swim about in drops of moisture on the leaves.

Buds have been infected by inoculation with the organisms. When the inoculations were carried out in spring, as the buds were expanding, infection arose without wounding, but the result was more pronounced when the buds were punctured at the time of inoculation (Expt. 26/4). When the inoculations were made in autumn (Expt. 26/30) infection arose only on those buds which had been mechanically injured as well as inoculated. In other experiments (not recorded here) inoculations in autumn on buds, some punctured, others uninjured, yielded negative results.

The results of Expt. 32/2 suggest that some strains of *Pseudomonas mors-prunorum* are more virulent than others. The most virulent strain in this experiment was one isolated from a leaf spot, and this offers clear evidence that the organism in the spots is able to produce cankers.

In those experiments in which the trees were kept under observation for two years or more after inoculation, the development of the branch lesions was completed during the summer of the year in which they appeared (32/2 and 32/6). When the inoculations were made in autumn, the lesions became noticeable in the following spring, but after that they extended no farther. If they had not girdled the branches by that time, callus developed round the edges of the lesions, which eventually became healed over. This conforms with what has been observed in natural infections ; in these the cankers usually cease to increase in size during the summer and the organisms in such cankers then die out. Attempts to isolate the organism in late summer have rarely been successful.

In the inoculations on branches, infection was followed by an exudation of gum (29/26, 29/27, 29/28). Mechanical injury to the branches (such as cuts through the bark), when not followed by infection, produced little or no gumming, whether bacteria had been applied to the wounds or not ; but when lesions developed, copious gum exuded. The question as to whether this gum is a result of bacterial action on the tissues of the cherry tree, or whether it is

merely the response of the tree to the interruption in the flow of sap, is outside the scope of this paper. The results do show, however, that bacterial canker is one of the causes of gumming in sweet cherries.

Although the experiments described show clearly that the organisms are definitely parasitic when the conditions are favourable for their development, in other experiments the results have been negative or inconclusive, and it would seem that here, as in many other plant diseases, the degree of infection following inoculation is greatly affected by the physiological condition of the trees, as influenced by weather and soil conditions. Wet weather is undoubtedly favourable for leaf and shoot infection, but the conditions favouring or checking infection of branches and stems have not yet been determined. Some information regarding nutrition in relation to stem infections on plum trees has been obtained (2), but similar experiments with cherry trees have not been attempted.

SUMMARY.

The organisms associated with bacterial lesions on sweet cherry trees in Britain have been found to be *Pseudomonas prunicola* and *P. mors-prunorum*, the bacteria causing similar diseases on plum trees.

Cultural tests for distinguishing the two organisms are described. For quick tests the organisms are grown in nutrient broth with 5 per cent. saccharose, for a colour difference in the turbidity of the resulting growth, and also in a nutrient agar with 5 per cent. saccharose, for a longevity test, *P. mors-prunorum* (but not *P. prunicola*) dying out within six days.

Inoculation experiments have shown that each organism is able to infect the various parts of a tree liable to attack. Attention is drawn to the relation between the stem canker and the leaf spot phases.

REFERENCES.

(1) *Aderhold, R.* and *Ruhland, W.* Der Bakterienbrand der Kirschbäume. Arb. a.d. Kaiserl. Biol. Anst. f. Land- und Forstwirtschaft, 1907, **5**, 293-340.

(2) *Beard, F. H.* and *Wormald, H.* Bacterial Canker of Plum Trees in Relation to Nutrition. Experimental Results in Sand Cultures. (With an Appendix by W. A. Roach.) E. Malling Res. Sta. Ann. Rep. for 1935 (May 1936), 146-54.

(3) *Griffen, F. L.* A Bacterial Gummosis of Cherries. Science (N.S.), 1911, **34**, 615-16.

(4) *Grubb, N. H.* Bacteriosis of Cherry Trees. Relative Susceptibility of Varieties at East Malling. Journ. Pom. & Hort. Sci., 1937, **15**, 25-34.

(5) *Wilson, E. E.* Symptomatic and Etiological Relations of the Canker and the Blossom Blast of Pyrus and the Bacterial Canker of Prunus. Hilgardia, 1936, **10**, 213-40.

(6) *Wormald, H.* Bacterial Diseases of Stone Fruit Trees in Britain. II. Bacterial Shoot Wilt of Plum Trees. Ann. Appl. Biol., 1930, **17**, 725-44.

(7) ———. Ibid. IV. The Organism causing Bacterial Canker in Plum Trees. Trans. Brit. Mycol. Soc., 1932, **17**, 157-69.

(8) ———. Ibid. VI. Field Observations on Bacteriosis of Sweet Cherry Trees. Journ. Pom. & Hort. Sci., 1937, **15**, 35-48.

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FIG. 1.

Cherry twig showing result of inoculating alternate buds through punctures. The inoculated buds and also those alternating with them show infection. (Exp. 26/4)

FIG. 2.

Portion of cherry twig showing result of inoculating alternate buds without wounding. The inoculated buds produced infected shoots (on left) and there is some infection on the centre shoot between them (on right) although it was not directly inoculated.

FIG. 3.

Control twig, buds punctured ; the leaves show mechanical injury only.

Fig. 4.

Cherry branches inoculated in November with *Pseudomonas prunicola*, showing the result in the following summer. The point of inoculation was about two inches above the base of the affected portion. (Exp. 29/28)

Fig. 5.

Portion of a branch seen in Fig. 4, showing the infected area and a mass of exuded gum.

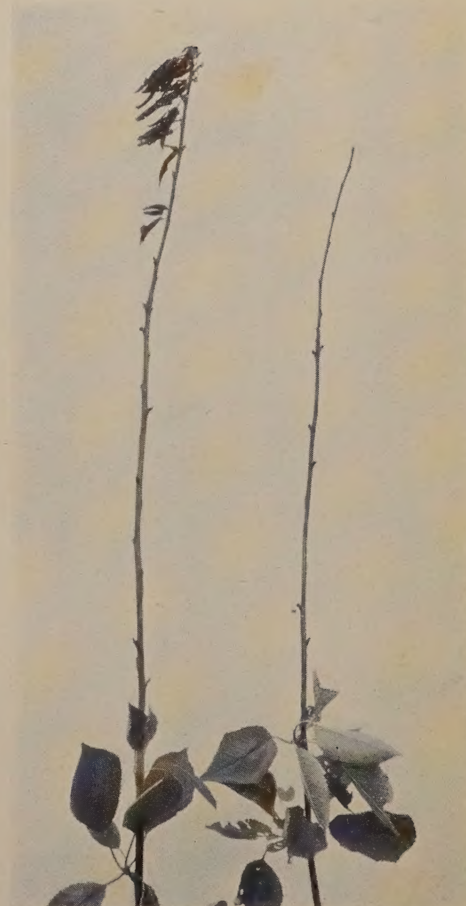

Fig. 6.

Cherry branches inoculated with *P. mors-prunorum*. The point of inoculation in each is about three inches above the base of the affected portion.